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Author Comments: April 11, 2018
Section Editor, BioDrugs Journal
Dear Dr. McNab
Thank you for the provisional acceptance of our review manuscript entitled: "Therapeutic targeting of the IL-4/IL-13 Signaling Pathway: In Allergy and Beyond".

We have addressed all of the comments that were raised by the Editor and believe that the manuscript is now suitable for publication in BioDrugs Journal.

I affirm that all authors concur with the submission of this manuscript and that the material presented in this work has not been previously reported elsewhere.

Sincerely,
Ariel Munitz, PhD

Response to Reviewers: Response to Editorial Comments
All of the comments were addressed in the main text and Table files as suggested by the Editor

Suggested Reviewers: Michael E Wechsler wechslerm@njhealth.org
Expert in anti-cytokine therapy especially in targeting type 2 cytokines such as IL-13, IL-5 and IL-4

Joshua Milner jdmilner@niaid.nih.gov
Expert in Type 2 immunity and associated diseases

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Expert in effector functions of IL-4 and IL-13
Therapeutic Targeting of the IL-4/IL-13 Signaling Pathway: In Allergy and Beyond

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Running title: IL-4/IL-13 therapeutics

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Key points:

- IL-4 and IL-13 have pivotal activities in type 2 immune responses
- IL-4 and IL-13 trigger their cellular responses by interacting with an overlapping and complex receptor signaling system comprised of the type 1 and type 2 IL-4 receptors
- Recent data highlight new and exciting roles for IL-4 and IL-13 in metabolism, bone resorption and cognitive behavior
- Therapeutic targeting of the IL-4/IL-13 pathway may be beneficial for patients suffering from allergic diseases such as atopic dermatitis, asthma and eosinophilic esophagitis
Abstract

Inflammation triggered by IL-4/IL-13 is mediated by IL-4 and IL-13 receptors that are present on multiple cell types including epithelial cells, smooth muscle, fibroblasts endothelial cells and immune cells. IL-4 exerts its activities by interacting with two specific cell surface receptors. One, designated the type 1 IL-4 receptor (IL-4R), the other, designated the type 2 IL-4R, a receptor complex that is also the functional receptor for IL-13. “Traditionally”, IL-4 and IL-13 have been studied in the context of T helper 2-associated immune responses (i.e. type 2 immunity). In these settings, IL-4, IL-13 and their cognate receptor chains display pivotal roles where IL-4 is considered an instigator of type 2 immune responses and IL-13 an effector molecule. Thus, therapeutic targeting of the IL-4/IL-13 pathway is under extensive research, mainly for the treatment of allergic diseases. Nonetheless, in addition to their roles in type 2 immune responses, recent data highlight key activities for IL-4 and IL-13 in additional settings including metabolism, bone resorption and even cognitive learning. This review summarizes the established knowledge that has accumulated regarding the roles of IL-4, IL-13 and their receptors in allergic diseases with emphasis on asthma, atopic dermatitis and eosinophilic esophagitis. We will further overview the pharmacological entities, which were designed and examined over the years targeting these cytokines and/or their receptors. Finally, we will briefly highlight the new roles for IL-4 and IL-13.
1. **IL-4 and IL-13, “Hallmark” Th2 Cytokines**

The 1980’s were groundbreaking years in B and T cell research. Among the prominent findings of that time was the discovery of interleukin-4 (IL-4) in 1982 and interleukin-13 (IL-13) in 1989 (1, 2). Since then, the roles of IL-4 and IL-13 in immunity have been gradually elucidated, leading to the current understanding that IL-4 and IL-13 are fundamental immune regulating cytokines of T helper 2 (Th2)-mediated immune responses with a complex signaling system and pleotropic functions. IL-4 was described as a key regulator of Th2 cell differentiation and a key factor involved in B cell isotype switching. Alongside, IL-13 was identified as an inhibitor of inflammatory cytokine production from T cells and an inducer of IgE class switching in B cells.

IL-4 and IL-13 are encoded by neighboring genes located on locus 5q31.1 and share several epigenetic regulatory elements (3, 4). Both cytokines share the same receptor system and signal predominantly through the same transcription factor, namely: signal transducer and activator of transcription 6 (STAT6) (5). The shared signaling complex likely accounts for the fact that IL-13 was initially considered as an IL-4-like cytokine with redundant functions. However, over the past years it has become clearly apparent that the two cytokines possess several roles that are non-redundant and that STAT6-independent signaling also contributes to IL-4-induced functions, specifically in myeloid cells (6, 7). While early studies have focused on the roles of IL-4 and IL-13 in Th2 immune responses, our knowledge regarding the effector functions of IL-4 and IL-13 has substantially increased and it is now clear that IL-4 and IL-13 play roles that are
far beyond “classical” Th2 immunity (e.g. allergy and parasite infections) with functions in metabolism, tissue regeneration, remodeling, cancer and even learning and memory (8). This review will summarize the current knowledge regarding IL-4 and IL-13 with emphasis on their cellular source, signaling system and roles in allergy and beyond. In addition, we will outline current pharmacological strategies targeting these cytokines and/or their receptors.

1.1. The Cellular Source of IL-4 and IL-13

Many different cell types are capable of secreting IL-4 and IL-13 in vitro. However, in vivo, the cellular source for these cytokines is restricted to distinct tissues at specific time points (9). The development of new genetic tools, such as IL-4 and IL-13 reporter mice, which enabled the visualization of cytokine-expressing and/or producing cells under homeostatic conditions and in response to inflammatory triggers, enhanced our understanding on the expression pattern of these cytokines in vivo. As illustrated in Figure 1A, in response to antigen presentation and in the presence of IL-4 from a cellular source that is yet unclear, CD4+ Th2 cells can secrete both IL-4 and IL-13, either in a coordinated manner in which both cytokines are secreted from the same cell or in an un-coordinated manner in which a single cytokine is secreted from distinct Th2 cells, producing either IL-4 or IL-13 (10, 11). Invariant NKT (iNKT) cells can secrete IL-4 (but not IL-13, Figure 1B) while group two innate lymphoid cells (ILC2) are the predominant source for IL-13 following exposure to alarmins such as IL-33 and to epithelial-derived cytokines such as IL-25 and TSLP (9, 12, 13)(Figure 1C). Eosinophils are a significant source for IL-4 during allergic responses and
helminth infections (9, 14, 15) (Figure 1B). Finally, basophils and mast cells can secrete IL-4 and IL-13 while T follicular helper (Tfh) cells have been shown to secrete IL-4 only (9, 15-19).

1.2. The IL-4 Receptor Signaling System

Although IL-4 and IL-13 share only 25% sequence identity, they have many overlapping functional properties (20-22). The basis for these overlapping functions stems from the fact that they share a common receptor subunit, and signaling pathway involving the Janus kinase (JAK):STAT6 pathway (23). The IL-4 receptor signaling system (Figure 2A) comprises two receptors, the type 1 IL-4 receptor (IL-4R) and the type 2 IL-4R (5, 24). The type 1 IL-4R is comprised of the IL-4Rα chain, which is paired with the common γ chain, while the type 2 IL-4R is comprised of the IL-4Rα chain paired with IL-13Rα1. IL-4 binds the IL-4Rα chain and therefore can signal both via the type 1 or type 2 IL-4Rs. In contrast, IL-13 binds IL-13Rα1 and can therefore signal via the type 2 IL-4R. Despite the fact that both IL-4 and IL-13 can utilize the type 2 IL-4R, they do so in a different manner (25). IL-4 binds IL-4Rα with high affinity (Kd~ sub-nanomolar) and the recruitment of either the common γ chain or IL-13Rα1 does not add to the affinity of the receptor. In fact, the recruitment of IL-13Rα1 to the pre-formed IL-4:IL-4Rα dimer is mediated by a low affinity interaction and is considered as a rate-limiting step. In contrast, IL-13 binds IL-13Rα1 with moderate affinity (Kd~ 30nM) but the recruitment of IL-4Rα to the pre-formed IL-13:IL-13Rα1 dimer increases the affinity of IL-13 to IL-13Rα1 to a picomolar level (25). Thus, IL-13 binding to IL-
13Rα1 favors the formation of the IL-13:IL-13Rα1:IL-4Rα receptor over the formation of IL-4:IL-13Rα1:IL-4Rα. An additional factor that contributes to the differential signals driven via the type 2 IL-4R by IL-4 and/or IL-13 is the relative abundance of each receptor chain on the cell surface. It is estimated that the number of IL-4Rα molecules on the cell surface is limited (~50-5000 molecules/cells). However, the number of IL-13Rα1 molecules on the surface of structural cells (e.g. epithelial cells, fibroblasts, endothelial cells, muscle cells) is relatively abundant (~5000-150000 molecules/cells) while its expression on B lymphocytes and monocytes is low and nearly absent on the surface of T lymphocytes (26-28). Collectively, unless IL-13Rα1 or the common γ chain are in substantial excess, only a portion of the IL-4:IL-4Rα complexes will achieve the capacity to signal. By contrast, the formation of the IL-13:IL-13Rα1:IL-4Rα complex is 25-fold more efficient than the formation of the IL-4:IL-13Rα1:IL-4Rα or the IL-4:γc:IL-4Rα complexes (25). Nonetheless, due to the lower affinity of IL-13 to IL-13Rα1, higher concentrations of IL-13 are needed in order to initiate signaling rather than IL-4 that will achieve maximum receptor signaling in much lower concentrations.

Cellular response to IL-4 or IL-13 via the type 1 or type 2 IL-4Rs are regulated also by the differential expression of the γc chain. Since the γc chain is predominantly expressed by immune cells, the type 1 IL-4R is widely expressed on immune cells of the lymphoid and myeloid lineage. In contrast, the type 2 IL-4R is widely expressed predominantly by non-hematopoietic cells such as epithelial cells and fibroblasts. Although the type 2 IL-4R is expressed by myeloid
cells it is almost completely absent on the surface of T and B cells (29). The
differential utilization of the type 2 IL-4R along with the differential cellular
expression of the type 1 and type 2 IL-4R, provides an elegant explanation for
the functional differences between IL-4, which is considered as an immune-
modulator cytokine and IL-13 that is considered as an effector cytokine. This also
provides an explanation for some of the non-redundant functions of the two
cytokines.

Adding complexity to this receptor signaling system, IL-13 can bind an additional
receptor, termed IL-13Rα2 (30) (Figure 2C). IL-13Rα2 is expressed in two forms;
a membrane-bound form (mIL-13Rα2) and a soluble form (sIL-13Rα2). mIL-
13Rα2 and sIL-13Rα2 bind IL-13 with high affinity (20-90pM), which is ~100-300
fold higher than the binding of IL-13Rα1 to IL-13. The exact role of IL-13Rα2 in
mediating IL-13 signaling is not fully understood. Due to the higher affinity of IL-
13Rα2 in comparison with IL-13Rα1, the sIL-13Rα2 is considered as a decoy
receptor, which can “capture” IL-13 and decrease the availability of this cytokine
to bind IL-13Rα1. However, the soluble form of IL-13Rα2 is not expressed in
humans (30). Thus, due to the lack of sIL-13Rα2 (and hence the lack of decoy
receptor), it is more likely to assume that human IL-13Rα2 is capable of
signaling. In support of this notion, human IL-13Rα2 was shown to signal in
several human monocytic cell lines (31) and in glioblastoma multiforme (32).

1.2.1. Signal Transduction Following IL-4R Engagement
Initiation of signal transduction by the IL-4 receptors requires receptor-associated kinase phosphorylation since neither of the IL-4R chains contains endogenous kinase activity. The predominant signaling cascade initiated by IL-4R engagement is the Jak:STAT signaling pathway. IL-4Rα associates with Jak1 and Jak2, the γc chain associates with Jak3 and IL-13Rα1 can associate with Jak2 and Tyk2 (Figure 2A-B). Following engagement of IL-4 or IL-13 to their respective receptors, phosphorylation of Jak-kinases leads to the phosphorylation and dimerization of STAT6. Subsequently, STAT6 translocates into the nucleus and induces the transcription of IL-4/IL-13-target genes.

Although STAT6 is the most prominent signaling pathway in IL-4 and/or IL-13-mediated responses, IL-4 can induce STAT6-independent signaling events via IL-4Rα as well. In fact, IL-4Rα can associate with insulin receptor substrate-1 and 2 (IRS1/2) that further recruits phosphoinositol-3 kinase (PI3K). IL-4-induced, PI3K-mediated signaling, was shown to regulate the survival of Th2 cells.

IL-4Rα is subjected to various levels of regulation. For example, biochemical studies have demonstrated that the IL-4Rα chain possesses an intrinsic immunoreceptor tyrosine-based inhibitory motif (ITIM), which can suppress IL-4 (and likely IL-13) signaling (33, 34). In addition, STIP1 homology and U-Box containing protein 1 (STUB1) interacts with IL-4Rα and targets it for degradation thus terminating IL-4 or IL-13 signaling (35) (Figure 2D). Furthermore, CD300f, is an Ig-superfamily receptor that mediates the engulfment of apoptotic cells by various myeloid cells in humans and mice. CD300f, at least in mice, has been
shown to directly associate with IL-4Rα and co-amplify IL-4Rα-induced STAT6 signaling events in response to IL-4 and IL-13 (36) (Figure 2E).

2. Functions of IL-4 and IL-13 in Allergy and Beyond

Initially, the roles of the IL-4 and IL-13 emerged from the studies of parasitic infections. Indeed, the immune response to helminth parasites is generally considered as a dominant type 2 immune response which leads to worm expulsion. As investigations into the functions of IL-4 and IL-13 deepened, the role of these cytokines in the allergic responses was also established. Currently, it is appreciated that IL-4 and IL-13 have roles in multiple non-allergic, non-Th2 governed conditions (Figure 3). In the following sections, we will overview the roles of IL-4 and IL-13 in various disease settings and assess the potential therapeutic value of targeting IL-4 and/or IL-13 in these settings.

2.1 “Traditional” Functions of IL-4 and IL-13: Helminth Infections

Helminth parasites are multicellular, macro pathogens, which are the most common parasitic infections worldwide affecting ~24% of the world’s population (WHO, 2017). Infections are mostly distributed in tropical and sub-tropical areas and mainly in third world countries. Infections with helminth parasites typically result in a robust type 2 immune response, which is characterized by the secretion of IL-4, IL-13, IL-5 and IL-10 and the accumulation of effector cells such as eosinophils, basophils, alternatively activated macrophages and mast cells. In addition, it is accompanied production of IgG1, IgG4 and IgE antibodies
This host response is more accurately referred to as “modified-type 2” response since it usually encompasses high levels of IL-10 and low levels of IgE (39). From an evolutionary point of view, it seems that mammals mount a “tissue injury-like” response following recognition of invading helminths and that the host response to these parasitic infections is directed towards tolerance and resistance. A Th2-dominant response is quite universal in helminth infections (40), and in fact, certain aspects of the host response towards helminth infections are completely dependent on IL-4Rα and STAT6 signaling. Certainly, worm expulsion, a process that involves mucus secretion, muscle hyper contractility and accelerated epithelial cell turnover, is highly dependent on IL-4Rα function in most helminth infection models (41-44). However, while a type 2 immune response is considered protective in re-infection with several helminth parasites, the resistance in individuals that display natural immunity to infections, is predominantly mediated by a type 1 response (45). In addition, there are several examples demonstrating that early infection with Schistosomes and Brugia malayi promotes a type 1 immune response that is progressively replaced by a type 2 immune response (46, 47). Taken together, it is currently understood that distinct mechanisms are involved in the elimination of diverse helminth species and even more so of distinct life cycle stages of the same species.

2.1.1. IL-4 and IL-13 in Allergic Disease: The Instigator and the Effector Cytokine of the Allergic Response

The allergic response can be divided into two major phases: the sensitization phase and the effector phase. For example, in asthma sensitization to a specific
allergen occurs in atopic individuals long before the appearance of disease symptoms such as wheezing, bronchoconstriction and airway inflammation. During this asymptomatic stage, antigen presentation by dendritic cells will induce the differentiation of naïve CD4+ T cells into Th2 cells, differentiation of B cells into IgE secreting plasma cells and the binding of IgE antibodies to FcεR1 receptors on the surface of mast cells and basophils. IL-4 is critical for these initial responses and is therefore considered as a key instigator of the allergic response (48-50). Subsequently, following repeated exposure to the allergen, IgE cross-linking on mast cells may occur (in allergen-sensitized patients) causing the secretion of histamine, arachidonic acid metabolites (e.g. prostaglandins, leukotrienes) and various chemokines that induce the transmigration of inflammatory cells, most importantly eosinophils. This late-phase response is predominantly regulated by IL-13 and characterized by the presence of eosinophils. Subsequently, increased IL-13 levels result in the manifestation of disease symptoms including epithelial cell hyperplasia, mucus hypersecretion and tissue remodeling (51-53), all of which can be directly mediated by IL-13. Accordingly, while many over-lapping functions for IL-4 and IL-13 are seen in the allergic response, IL-4 is generally considered more as the initiator of the allergic response and IL-13 as the effector cytokine responsible for clinically relevant disease symptoms.

2.1.2. Role in Asthma
The critical involvement of IL-4 and IL-13 in asthma onset and progression was clearly established early in the research of type 2 immune responses. Nonetheless, asthma is acknowledged as a heterogeneous disease encompassing several different phenotypes including eosinophilic asthma, neutrophilic asthma and mixed granulocytic asthma (54). A hallmark Th2-mediated response, which includes high eosinophilic count in the blood and bronchoalveolar (BAL) fluid and responsiveness to corticosteroids is currently referred to as eosinophilic asthma, which can be found in allergic and non-allergic individuals (55). This asthma endotype, is often associated with increased expression of IL-4 and IL-13 and therefore, targeting IL-4 and IL-13 during eosinophilic asthma could prove to be clinically significant (see Table 1 and section 3 below: “Therapeutic Targeting of the IL-4/IL-13 Pathway”).

2.1.3. Role in Atopic Dermatitis

Atopic Dermatitis (AD) is a chronic, relapsing, inflammatory disease of the skin. AD may precede the development of asthma and/or other allergic disorders in a phenomenon termed the “atopic march” (56), where skin sensitization in early childhood eventually may evolve into allergic reactions in other organs such as the lung. Nonetheless, AD may occur without concomitant allergic sensitization and may not associate with an increased risk of asthma (57). The common features of AD are defective skin barrier, atopy, elevated Th2 response and predisposition to viral skin infections (58). AD patients are classified into two groups; extrinsic AD (approximately 80-90% of patients) manifests with skin inflammation accompanied by high IgE levels in the serum while intrinsic AD has
no allergen specific IgE or total IgE antibodies found in their blood or skin and is considered non-allergic AD (59). IL-13 expressing CD4+ Th2 cells and protein levels are higher in acute skin lesions than in chronic skin lesions. In general, IL-13 is expressed to a greater extent than IL-4 during disease progression (60). In addition, while high IL-13 levels and eosinophils counts are characteristic of extrinsic AD, lower IL-13 levels and eosinophil counts are found in intrinsic AD patients along with elevated Th17 and Th22 response (60, 61). Actually, IL-13 levels are directly correlated with disease severity and flares (62). Several studies assessing the roles of IL-4 and IL-13 in the etiology of AD highlighted their involvement in keratinocyte activation, leading them to secrete T cell chemokine attractants. Furthermore, IL-4- and IL-13-activated keratinocytes displayed decreased barrier function. This is likely achieved by the ability of IL-4 and IL-13 to attenuate fibroblast and keratinocyte extracellular matrix proteins, such as matrix metalloproteinases (MMPs) and adhesion molecules expression (63). Taken together, neutralizing the activities of IL-4 and IL-13 in extrinsic AD patients might facilitate better disease management.

2.1.4. Role in Eosinophilic Esophagitis

EoE represents a chronic, local immune-mediated esophageal disease, characterized clinically by symptoms related to esophageal dysfunction and histologically by eosinophil-predominant inflammation. Importantly, systemic and/or local causes for esophageal eosinophilia should be excluded (64). Symptoms of EoE include dysphagia, food impaction, vomiting, pain and failure
to thrive (65). In accordance with the food allergen-driven nature of this disease, most patients will benefit greatly from dietary changes and antigen avoidance that can be achieved by elemental diet (66). However, while the majority of EoE patients will present IgE-mediated hypersensitivity to food allergens, non-IgE mediated reactions (delayed type, T cell mediated) are also recognized in the pathogenesis of EoE (67, 68). Thus, it is now appreciated that both allergen specific IgE-mediated and T-cell mediated allergic sensitization are independent mechanisms underlying the Th2 immune response in EoE. Key involvement for IL-13, TSLP and eotaxin-3 has been suggested in mediating the accumulation of eosinophils in EoE. For example, IL-13 is capable of inducing a remarkably similar epithelial cell transcriptome signature in mice (e.g. in experimental EoE) and human EoE patients. Furthermore, IL-13 is a potent inducer of eotaxin-3 in human esophageal epithelial cells, which is responsible for eosinophil accumulation in the esophagus (69, 70). Furthermore, IL-13 was shown to be involved in reduced barrier function, fibrosis, epithelial cell hyperplasia and angiogenesis during EoE (71-74). Taken together, anti-IL-13 therapy might be beneficial for the management of EoE. Nonetheless, decreasing IgE levels and/or Th2 sensitization by targeting IL-4 may be beneficial as well.

2.2. IL-13: The Two-Faced Cytokine in Tissue Remodeling

While tissue remodeling is an endpoint consequence of many different diseases stemming from multiple etiologies (e.g. parasitic infections, allergic disorders, cardiovascular disease, inflammatory bowel disease and cancer), in some diseases
the actual tissue damage, caused by fibrosis, is considered to be the disease itself. These diseases are considered “hallmark” fibrotic diseases. For example, two of these diseases that affect the lungs are chronic obstructive pulmonary disease (COPD) and idiopathic pulmonary fibrosis (IPF). While the pro-fibrogenic role of the Th2 cytokines, IL-4 and IL-13, is well established in parasitic infections and allergic diseases, the function of these cytokines in IPF seems to be more complex than previously appreciated.

IPF is an interstitial lung disease affecting 3-6 out of 100,000 people in the general population and is currently on the rise (75). IPF is characterized by a restrictive pattern of lung volume abnormality that is associated with impaired diffusion capacity following exposure to an idiopathic irritant (76). Although IPF is a relatively rare disease it is deadly with a median survival rate of less than three years (77). For many years it was accepted that chronic inflammation was the underlying cause of pulmonary fibrosis, nonetheless, the finding that IPF patients do not respond to glucocorticoid therapy challenged the contribution of ‘inflammation” per se, to IPF pathogenesis. The underlying pathology in IPF is apoptosis of epithelial cells, activation of fibroblasts and accumulation of extracellular matrix (ECM) (78), likely in response to repetitive micro injuries. Thus, it is currently hypothesized that during IPF, aberrant wound healing responses occurs in reaction to chronic stimuli in the lungs (78). IL-13 can regulate fibrosis in various ways, it can modulate fibroblast functions directly or through the regulation of TGF-β. In addition, IL-13 can promote synthesis of extracellular matrix proteins (ECM) and can promote alternative activation of macrophages, which are associated with fibrosis. Thus, IL-13 may
have a role in the pathogenesis of IPF (23, 79). Indeed, IL-13 levels were shown to be increased in IPF patients and fibroblasts isolated from IPF patients were hyper-responsive to IL-13 treatment (80, 81). However, the involvement of IL-13 in the pathogenesis of IPF remains controversial. IPF patients frequently present a type 1 pro-inflammatory cytokine profile rather than a type 2 cytokine profile. Furthermore, several studies report an IL-13 and IL-13Rα1 independent fibrosis in mouse models of pulmonary fibrosis. Finally, bleomycin-treated Il13ra1−/− mice displayed increased pathology likely due to a protective role for IL-13Rα1 in epithelial cells (82-84). Collectively, the involvement of IL-13 in the etiology of IPF and the possible targeting of this cytokine for therapy remain to be further investigated and tested by clinical trials.

3. Therapeutic Targeting of the IL-4/IL-13 Pathway

Over a dozen different therapeutic entities including; mutated ligands, soluble receptors, and antibodies have been developed in past years all aimed at targeting IL-4, IL-13 or IL-4Rα (Table 2). Based on in vitro and in vivo animal models, therapeutic targeting of IL-4/IL-13 held great promise. Nonetheless, early studies evaluating IL-4 and IL-13 targeting therapies showed disappointing results, mainly due to lack of efficacy. Importantly, dual IL-4/IL-13 neutralization by dupilumab, which showed promising results in clinical trials, has recently been approved by the FDA for the treatment of AD clinically validating the importance of this pathway in the pathophysiology of allergic diseases. In this section, we will review current and past therapeutics targeting the IL-4/IL-13 pathway.
### 3.1. Entities Targeting IL-4

Two entities were previously developed for targeting IL-4. Altrakincept (Immunex/Amgen) is a recombinant soluble IL-4Rα protein that inhibits IL-4 signaling by competing with the membrane receptor. Two phase I/II studies enrolling moderate asthmatics, either dependent or independent on inhaled corticosteroids (ICS) were conducted (85, 86). Interestingly, in these studies, Altrakincept was delivered via the inhalation route and had a serum half-life of approximately one week. In both trials, an improvement of lung functions (mainly FEV$_1$) was shown. However, no decrease in blood eosinophils or total serum IgE levels was observed and no additional studies were performed. A second entity neutralizing IL-4 (human IgG1, pascolizumab, GlaxoSmithKline) was under a phase II study on mild asthmatics. To date, the outcomes of this study were not reported (NCT00024544).

### 3.2. Entities Targeting IL-13

In agreement with the predominant effector functions of IL-13 in the effector stage of the allergic response, significantly more efforts were devoted to the therapeutic targeting of IL-13 (versus IL-4). Several different entities were tested clinically. Among those, studies using tralokinumab (MedImmune/AstraZeneca) and lebrikizumab (Genentech) were initially most promising.

Tralokinumab is a fully human anti-IL-13 IgG4 antibody that was tested in severe asthmatics, atopic dermatitis patients as well as IPF patients. Although Tralokinumab showed promising results in early phase II asthma trials (87, 88), but
according to topline results, which were announced by AstraZeneca and a
summary of the STRATOS2 clinical trial (NCT02194699), failed to meet the primary
end points of reduced annual exacerbations or reduction in the use of oral
corticosteroids (OCS) in phase III studies.

Lebrikizumab, a fully humanized IgG4 mAb, also failed to improve clinical outcomes
in phase II trials (89-94). Interestingly, in the two LAVOLTA phase III trials, despite
meeting a statistically-significant reduction in exacerbations rate, which was the
primary study end-point, the authors concluded that the overall effect was not
clinically meaningful (95). Collectively, these data raise the question whether the
selection of this primary end point was indeed indicative of efficacy and whether
better stratification of patients was required.

The combined data emerging from strategies targeting a single cytokine and
especially of IL-13 highlight the importance of IL-13 in asthma. Nonetheless, the
data raise the notion that inhibition of IL-4 or IL-13 alone is not sufficient to manage
clinically significant aspects of the disease. Thus, better strategies and biomarkers
that will predict patient responsiveness to therapy should be developed.

3.3. Dual Targeting of IL-4/IL-13

Following inconsistent success with single cytokine targeting, significant
advancements in the treatment of allergic diseases came from the dual targeting of
IL-4 and IL-13. Four different drugs were developed so far for the targeting of both
cytokines; a mutant IL-4 protein which antagonizes IL-4Rα (Pitrakinra, Aerovance),
antibody combination of anti-IL-4 and anti-IL-13 (QBX258, Novartis) and two anti-IL-4Rα antibodies (AMG-317, Amgen and dupilumab, Regeneron/Sanofi).

Dual cytokine targeting via dupilumab appears to be quite effective in allergic diseases and therefore dupilumab was rapidly termed an “asthma hit”. Dupilumab is a human IgG4 antibody targeting IL-4Rα, which has been primarily studied in the context of AD with great potency and was approved by the Food and Drug Administration (FDA) in March 2017. Nonetheless, dupilumab shows promising results in, asthma, chronic rhinosinusitis with nasal polyposis, and EoE. As of January 1st, 2018, dupilumab has been registered to more than 30 trials of which 17 are completed with relative success.

3.3.1. Dupilumab in Atopic Dermatitis

Four I/II phase combined studies, evaluated the efficacy and safety of dupilumab in moderate-to-severe atopic dermatitis (AD) patients using topical corticosteroids (TCS) and calcineurin inhibitors. The results of these studies were published simultaneously (96), demonstrating improvements in Eczema Area and Severity Index (EASI)-50, IGA, pruritus, and incidence of skin infections, while reducing TCS. Outcomes of these studies also suggested that clinical efficacy of dupilumab was independent of serum IgE levels as clinical benefits were observed at 4 weeks of treatment, whereas serum IgE levels were reduced only after 12 weeks. A subsequent phase IIb trial in moderate-to-severe inadequately controlled AD patients with topical medications, evaluated several dose regimens of dupilumab (300mg Q1W, 300mg Q2W, 300mg Q4W, 200mg Q2W, or 100mg Q4W). All dose
regimens significantly improved EASI, SCORing Atopic Dermatitis (SCORAD), and pruritus scores over placebo in a dose-dependent fashion (97). In addition, the phase III replicate studies SOLO 1 and SOLO 2 repeatedly showed the superiority of dupilumab over placebo with clinically significant improvements in IGA, EASI, pruritus, anxiety and depression (HADS), and quality of life (DLQI). Common adverse events in the dupilumab treated patients were injection-site reactions and conjunctivitis (98). An additional phase III study, CHRONOS, evaluated the efficacy and safety of dupilumab as an add-on therapy to TCS during a 52-week period. Dupilumab met the co-primary end points, IGA and EASI-75 by Week 16, and improvements in pruritus and SCORAD were observed as well, while reducing the use of TCS. These results were maintained for 52 weeks with comparable adverse events were reported in the dupilumab and placebo groups. Nonetheless, injection-site reactions and conjunctivitis were more common in patients treated with dupilumab (99). In March 2017, the FDA approved the use of dupilumab in adult atopic dermatitis patients whose disease is inadequately controlled with topical prescription medications. Dupilumab is also under evaluation for pediatric (aged 6-12) and adolescent (aged 12-18) AD patients. To date, one of these studies has been completed (NCT02407756) and the other one is still active (NCT03054428) while two additional studies involving patients under 18 years of age are recruiting (NCT02612454, NCT03345914). Finally, a phase I study is currently evaluating an auto-injector device in adults suffering from moderate-to-severe AD (NCT03050151).
3.3.2. Dupilumab in Asthma

Multiple phase II trials demonstrated the safety and efficacy of subcutaneous dupilumab in asthma. In a study of persistent eosinophilic asthma, subcutaneous dupilumab improved exacerbations rate by 87% and FEV$_1$ by 0.27L compared with placebo. These effects were sustained even after inhaled corticosteroids (ICS) and Long-Acting Beta Agonist (LABA) withdrawal. Moreover, dupilumab significantly reduced serum IgE, eotaxin, CCL17, and nitric oxide (NO) levels (100). A multinational study in an uncontrolled asthma population evaluated 4 dose regimens of dupilumab as an add-on therapy over 24 weeks. By Week 12, dupilumab improved FEV$_1$ and exacerbations rate in 3 of the dose regimens (200mg Q2W, 300mg Q2W, and 300mg Q4W)(101). Another study is ongoing to evaluate the effect of dupilumab on airway inflammation in persistent asthma (NCT02573233). Topline results from two phase III studies have been released by Regeneron & Sanofi. In the QUEST study on persistent asthma patients (NCT02414854), dupilumab met the two primary end points, FEV$_1$ (0.13L increase) and exacerbations rate (46% decrease) over placebo in the overall population. Patients with medium or high eosinophil counts showed greater benefits with 0.24L increase in FEV$_1$ and 67% decrease in exacerbations rate in the eosinophil-high subgroup. In the VENTURE study (NCT02528214), dupilumab was evaluated as an add-on therapy to the standard of care in severe, oral corticosteroids (OCS)-dependent asthma. At 24 weeks, dupilumab reduced the use of OCS in the overall population, and, despite reducing OCS, exacerbations rate was reduced by 59% and FEV$_1$ was increased by 0.22L compared with placebo. In eosinophil-high patients, dupilumab decreased
exacerbations rate by 71% and increased FEV\textsubscript{1} by 0.32L. Most impressively, half of dupilumab-treated patients have completely eliminated their use of OCS. Full publication of peer-reviewed data from QUEST and VENTURE is eagerly awaited. The long-term efficacy and safety of dupilumab in pediatric patients is currently being assessed (NCT02948959).

3.3.3. *Dupilumab in chronic rhinosinusitis and nasal polyps*

In a single phase II study that was conducted in chronic rhinosinusitis and nasal polyposis (CSwNP) patients, dupilumab was evaluated as an add-on therapy to mometasone furoate nasal spray. Dupilumab met the primary end point of improved nasal polyp score. Notably, larger benefits were observed in patients with co-morbid asthma, a subset of which also showed FEV\textsubscript{1} and Asthma Control Questioner (ACQ) improvement. Common side effects included nasopharyngitis, injection-site reactions, and headaches (102). Two phase III studies, SINUS-24 and SINUS-52 are ongoing to evaluate dupilumab as an add-on therapy in CSwNP.

3.3.4. *Additional entities targeting IL-4R\textalpha*  

Pitrakinra and AMG-317 represent additional and distinct therapeutic entities targeting IL-4R\textalpha. Pitrakinra is a recombinant mutated IL-4 variant that binds IL-4R\textalpha and prevents its complexing with γc and IL-13R\textalpha1 chains, thereby neutralizes signaling of both the type 1 and type 2 IL-4Rs (103). AMG-317, a monoclonal antibody to IL-4R, was tested in a phase II trial using several doses in moderate-to-
severe asthmatics with disappointing results (104). This trial tested 75, 150, or 300mg subcutaneous injection of AMG-317 compared with placebo in moderate-to-severe asthma. None of the dose regimens showed improvement of ACQ, which was the primary end point, or key secondary end points in the overall population. Nonetheless, a pre-planned analysis revealed clinically significant improvements in patients displaying the highest disease severity (105). These results are surprising especially in light of dupilumab's success to meet similar clinical endpoints. A plausible explanation for the different activities of these drugs may be due to the high clearance of AMG-317. A following PK analysis (105) identified a high clearance rate for AMG-317 of 35.0 mL/hr, which is, for instance, ~3 times higher than tralokinumab's clearance rate (8mL/hr)(106). Therefore, AMG-317 may have not sufficiently blocked IL-4 and IL-13 in this trial. This hypothesis was not assessed during the trial.

3.3.5. Dual IL-4/IL-13 targeting: Future perspectives

To date, the majority of patients that were recruited for clinical trials assessing therapeutics targeting IL-4/IL-13, were selected based on peripheral blood eosinophil counts and serum IgE levels. Given that complex disease phenotypes are observed in patients (presence or lack of IgE secretion, elevation or no change in eosinophil numbers, dependency or independency on corticosteroids and more) as well as the distinct asthma endotypes which have been characterized, it is reasonable to assume that better patient stratification according to the inflammatory cell composition, selected biomarkers and resistance to
corticosteroids would enable “tailored” treatment that might yield better clinical outcomes than those observed thus far. Moreover, the clinical benefit, which was observed in patients that have been treated with dupilumab raise the notion that dual targeting of IL-4 and IL-13 signaling may be superior to single cytokine strategies by targeting IL-4 or IL-13. It would be interesting to assess whether targeting IL-13Rα1, which can block IL-4 and IL-13 binding and subsequent signaling via the type 2 IL-4 receptor, would also prove beneficial.
4. **New Roles for IL-4 and IL-13: From the Bone to the Brain**

Owing to the development of new mouse models and technologies, that enable to assess the roles of IL-4, IL-13 and their receptor chains, it has become increasingly apparent that IL-4 and IL-13 have additional activities, which are not restricted to allergy and parasitic infections.

4.1.1. **IL-4 and IL-13 in Metabolism**

The structure and homeostasis of the adipose tissue is tightly regulated by the immune system. Under homeostatic conditions, the lean white adipose tissue is associated with an anti-inflammatory Th2-oriented environment. It is mainly populated with alternatively-activated macrophages, eosinophils, ILC2 cells and T regulatory cells. Furthermore, the cytokine milieu associated with the lean tissue is characterized by TGF-β, IL-10, IL-4 and IL-13 (107, 108). Obesity is a risk factor for numerous metabolic diseases that are collectively termed the metabolic syndrome. These diseases include type 2 diabetes, non-alcoholic liver disease, heart disease and cancer (109). Interestingly, low-grade chronic inflammation is an underlying pathology of obesity-associated metabolic syndrome. Under obese-conditions, the adipose tissue switches from an anti-inflammatory environment, to a pro-inflammatory one (110, 111) where the expression of TNF-α, IL-1β, IL-6 and IL-8 (all of which have been implicated in mediating insulin resistance) are increased (111, 112). It appears eosinophil-macrophage cross-talk is a major axis in glucose metabolism and insulin resistance. Recently, a new paradigm has emerged where in the adipose tissue eosinophils provide IL-4 to sustain the alternatively activated state of resident...
macrophages which secrete catecholamines to promote metabolic homeostasis (111, 113). Consequently, eosinophil-derived IL-4 is in the center of an immune-metabolic axis that forestalls obesity and glucose tolerance by promoting energy expenditure or by mechanisms yet to be defined (114). Upon increased caloric intake, eosinophils and subsequently eosinophil-derived IL-4 levels are decreased in the adipose tissue. Consequently, the adipose tissue is inflamed and becomes prone to metabolic disorders (113, 114). Thus, a tight balance between a Th1 and a Th2 environment is necessary for maintaining the homeostasis of the adipose tissue and to avoid adipose tissue fattening. Finally, IL-13 was shown to directly regulated glucose metabolism in the liver, and type 2 diabetics were shown to have lower IL-13 levels in the serum and skeletal muscle cells (115, 116). Taken together, it is possible that enhancing “protective” signaling by IL-4 and/or IL-13 could be used to control glucose levels and to treat type 2 diabetes. Nonetheless, strategies aimed at increasing IL-4 and/or IL-13 signaling should be done with caution due to the co-morbidities that are associated with metabolic diseases. For example, obese asthma patients exhibit increased release of leptin that primes and enhances eosinophil responses towards eotaxin (117). Thus, enhancing IL-4 and/or IL-13 signaling, which increase chemokine levels (118), may actually favor the migration and presence of eosinophils into the lungs and thereby promote a deleterious effect.

4.1.2. Th2 Cytokines in Bone Resorption
The bone is a dynamic tissue, where bone formation and bone resorption processes are tightly controlled to preserve skeletal size, shape, and structural integrity as well as mineral homeostasis (119). Bone resorption is an important process, which if un-regulated, can result in significant pathologies such as osteoporosis (120). Osteoclasts are myeloid cells of the monocyte/macrophage lineage that are exclusively responsible for bone resorption. In past years, it has become clear that IL-4 is an important regulator of bone resorption. IL-4 can directly suppress the differentiation of bone-marrow precursors to osteoclasts, and can suppress bone resorption by osteoclasts (121-123). Indeed, IL-4 levels were found to be lower in postmenopausal women with low bone mineral density than in women with normal bone mineral density and in osteopenic women (124). Thus, enhancing IL-4 levels could help treat osteoporosis by reducing osteoclastogenesis and bone resorption. However, strategies enhancing IL-4 and/or IL-13 signaling should be carefully examined due to possible unwarranted side effects such as systemic or local eosinophilia.

4.1.3. The Role of Th2 Cytokines in Cognitive Brain Functions

The brain is generally perceived to be shielded from immune cells. Yet, the importance of immune cells, mainly T cells, in the maintenance of brain integrity has been recently described. Cognitive decline, accompanying ageing and neurodegenerative diseases such as Alzheimer’s disease and multiple sclerosis, are associated with an increase in inflammation. This inflammatory state is skewed towards a Th1 phenotype with increased expression of IL-6, IL-1β and IL-18.
especially in the hippocampus (125). In contrast, the effect of a Th2-skewed inflammation on cognitive functions has been found to be protective. Meningeal Th2 cell numbers are increased following visuospatial learning and memory tasks as measured using a Morris water maze (MWM) test (126). IL-4 and IL-13, which are secreted from these Th2 cells, are able to stimulate astrocytes to produce brain-derived neurotrophic factor (BDNF) (126, 127). T cell deficient mice, Il4 deficient mice and Il13 deficient mice display severe cognitive impairment in learning tasks in the MWM test (126-128). In addition, IL-13Ra1 expression on dopaminergic neurons was shown increase their susceptibility to oxidative stress-mediated damage thereby contributing to their preferential loss in Parkinson’s disease. Interestingly, the Il13ra1 gene lies on the X chromosome within the PARK12 locus of susceptibility to Parkinson’s disease (129). Taken together, these studies establish an intriguing new role for IL-4 and IL-13 during learning and memory and highlight the possible involvement of these cytokines during neurodegenerative diseases.

5. Summary

IL-4 and IL-13 are hallmark Th2-immunity regulating cytokines with fundamental roles in the initiation and progression of several diseases, some of which are “hallmark” Th2-associatesd diseases while others far from being “classical” Th2-immune disorders. These two cytokines show complex, pleotropic functions in disease pathophysiology. While intensive research has established the importance of both IL-4 and IL-13 in the etiology of the allergic response,
therapeutic targeting of the IL-4/IL-13 pathway has proven less productive than expected. Explanations to this conundrum could come from differences between mouse models and human diseases, from patient classification and from the lack of monitoring mechanistic aspects of treatment in clinical trials. For example, the levels of IL-4 and/or IL-13 and/or IL-4R chains are almost never assessed in patients before, during, or after treatment with anti-IL-4/IL-13 therapy. Sub-classification of patients according to cytokine or receptor levels along with IgE levels, eosinophil counts and additional biomarkers could help the interpretation of clinical results and enable a patient-tailored treatment.

Finally, the clear involvement of the IL-4/IL-13 pathway in newly appreciated conditions highlights the possible therapeutic potential of this pathway in non-allergic disease settings and could prove fruitful in the future. Importantly, while treatment of Th2-associated diseases such as asthma, EoE and AD would require the inhibition/neutralization of IL-4 and/or IL-13 function, treatment of other diseases such as osteoporosis, diabetes or neurodegenerative diseases might require the enhancement of IL-4 and/or IL-13 activities, depending on the specific function of the pathway in disease pathophysiology.
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Conflict of interest declaration

Ariel Munitz is scientific consultant for GlaxoSmithKline, Compugen and Augmanity Nano LTD. DKA is an employee of Augmanity Nano LTD. IB and AB declare no conflict of interest.
References


**Figure legends**

**Figure 1. Cellular sources for IL-4 and IL-13 in-vivo**

Activation of T follicular helper cells (Tfh) in secondary lymphoid organs with antigen (in the presence of IL-4 from a yet unknown cellular source) induces them to produce IL-4 (A). Tfh-derived IL-4 is critical for initiating B cell-dependent humoral responses. During Th2 immune responses, naïve T helper cells, in secondary lymphoid organs and/or peripheral tissues, will be differentiated into a Th2 state following antigen presentation by antigen-presenting cells (mainly dendritic cells) in the presence of IL-4. This antigen-dependent stimulation is responsible for secretion of significant amounts of IL-4 and IL-13 establishing Th2 cells a major source for both cytokines (A). In peripheral tissues, diverse Th2-associated stimuli including IL-4, IL-13, IL-3, IL-5, alarmins (e.g. IL-33) and epithelial cell-derived cytokines (e.g. IL-25 and TSLP) can stimulate IL-4 secretion (but not IL-13) from iNKT cells, eosinophils and mouse basophils (human basophils were reported to also secrete IL-13) (B). Mast cells can secrete IL-4 and IL-13 following IgE cross-linking or following stimulation with IL-33 (B). ILC2 cells are a significant source for IL-5, an eosinophil priming and activation factor, which contributes to eosinophil-derived IL-4 secretion (C). ILC2 cells are also a major source for IL-13 following stimulation with alarmins and especially with IL-33 (C). Collectively, the induction of an IL-4/IL-13 Th2 response regulates the allergic immune-response (including mucus production, smooth muscle contraction and airway hyper-responsiveness) and supports anti-helminth immunity. IL- Interleukin; Th2- T helper type 2; TSLP- Thymic stromal lymphopoietin
IL-4 and IL-13 signal via a complex network of receptors (R). These receptors comprise of several receptor chains, which upon ligand binding dimerize and form the type 1 IL-4R and the type 2 IL-4R (A). The type 1 IL-4R comprises the IL-4Rα chain along with the common γ chain (γc). The type 2 IL-4R (B) comprises the IL-4Rα chain along with the IL-13Rα1 chain. IL-4 binds with high affinity IL-4Rα and therefore can signal via either the type 1 or type 2 IL-4R’s. In contrast, IL-13 binds IL-13Rα1 and can therefore signal only via the type 2 IL-4R. In addition, IL-13 can also bind IL-13Rα2 with high affinity. IL-13Rα2 functions as a decoy receptor for IL-13 signaling in mice due to its availability in soluble form (C). However, in humans IL-13Rα2 is expressed in a membrane bound form and can this elicit potential signaling via ERK-1/AP-1 dependent pathways(C). Following ligand binding to either the type 1 or type 2 IL-4R’s the tyrosine phosphorylation of Irs1/2 and Jak1 occurs. In addition, the type 1 IL-4R can recruit Jak3 and the type 2 IL-4R can recruit Jak2 and Tyk2. Finally, the phosphorylation and activation of signal transducer and activator of transcription 6 (STAT6) occurs and subsequently IL-4/IL-13-dependent genes are transcribed. In addition, following IRS1/2 phosphorylation, PI3K can be recruited and phosphorylated, enabling STAT-6-independent signaling. IL-4Rα signaling may be regulated by various know mechanisms; 1) IL-4Rα contains an intracellular intrinsic ITIM domain, which can suppress IL-4Rα signaling by recruiting phosphatases; 2) Recruitment of the chaperon STUB1 (D) marks IL-4Rα for
degradation; and 3) CD300f, an Ig-superfamily receptor, can physically associate with IL-4Rα (at least in mice) and acts to amplify IL-4 and IL-13-induced effects via the type 1 and type 1 IL-4R’s (E). IRS1/2- Insulin receptor substrate, JAK-Janus kinase; STUB1- STIP1 Homology And U-Box Containing Protein 1; TYK-tyrosine kinase, SHP- Src homology region 2 domain-containing phosphatase-; sIL-13Rα2- soluble IL-13Rα2; mIL-13Rα2- membrane IL-13Rα2, PI3K-Phosphatidylinositol-4,5-bisphosphate 3-kinase; STAT6- Signal transducer and activator of transcription 6.
Figure 3. Pleotropic function of IL-4 and IL-13

IL-4 and IL-13 play distinct yet pleotropic functions in various diseases. While helminth infections are considered as hallmark Th2-associated diseases, it is becoming apparent that Th1 cytokines are involved in early infection events. In asthma IL-4 is responsible for the initiation of the allergic response and IL-13 for later chronic events leading to tissue remodeling and fibrosis. IL-13 is the main regulator of eosinophilic esophagitis and is also considered as a main pro-fibrotic factor in idiopathic pulmonary fibrosis (IPF). In contrast, the process of bone resorption is mainly regulated by IL-4 and both IL-4 and IL-13 are key regulators of atopic dermatitis, adipose tissue homeostasis and glucose metabolism and brain cognitive functions such as learning and memory.
Therapeutic Targeting of the IL-4/IL-13 Signaling Pathway: In Allergy and Beyond

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Key points:

- IL-4 and IL-13 have pivotal activities in type 2 immune responses
- IL-4 and IL-13 trigger their cellular responses by interacting with an overlapping and complex receptor signaling system comprised of the type 1 and type 2 IL-4 receptors
- Recent data highlight new and exciting roles for IL-4 and IL-13 in metabolism, bone resorption and cognitive behavior
- Therapeutic targeting of the IL-4/IL-13 pathway may be beneficial for patients suffering from allergic diseases such as atopic dermatitis, asthma and eosinophilic esophagitis
Abstract

Inflammation triggered by IL-4/IL-13 is mediated by IL-4 and IL-13 receptors that are present on multiple cell types including epithelial cells, smooth muscle, fibroblasts, endothelial cells, and immune cells. IL-4 exerts its activities by interacting with two specific cell surface receptors. One, designated the type 1 IL-4 receptor (IL-4R), the other, designated the type 2 IL-4R, a receptor complex that is also the functional receptor for IL-13. “Traditionally”, IL-4 and IL-13 have been studied in the context of T helper 2-associated immune responses (i.e. type 2 immunity). In these settings, IL-4, IL-13 and their cognate receptor chains display pivotal roles where IL-4 is considered an instigator of type 2 immune responses and IL-13 an effector molecule. Thus, therapeutic targeting of the IL-4/IL-13 pathway is under extensive research, mainly for the treatment of allergic diseases. Nonetheless, in addition to their roles in type 2 immune responses, recent data highlight key activities for IL-4 and IL-13 in additional settings including metabolism, bone resorption and even cognitive learning. This review summarizes the established knowledge that has accumulated regarding the roles of IL-4, IL-13 and their receptors in allergic diseases with emphasis on asthma, atopic dermatitis and eosinophilic esophagitis. We will further overview the pharmacological entities, which were designed and examined over the years targeting these cytokines and/or their receptors. Finally, we will briefly highlight the new roles for IL-4 and IL-13.
1. **IL-4 and IL-13, “Hallmark” Th2 Cytokines**

The 1980's were groundbreaking years in B and T cell research. Among the prominent findings of that time was the discovery of interleukin-4 (IL-4) in 1982 and interleukin-13 (IL-13) in 1989 (1, 2). Since then, the roles of IL-4 and IL-13 in immunity have been gradually elucidated, leading to the current understanding that IL-4 and IL-13 are fundamental immune regulating cytokines of T helper 2 (Th2)-mediated immune responses with a complex signaling system and pleiotropic functions. IL-4 was described as a key regulator of Th2 cell differentiation and a key factor involved in B cell isotype switching. Alongside, IL-13 was identified as an inhibitor of inflammatory cytokine production from T cells and an inducer of IgE class switching in B cells.

IL-4 and IL-13 are encoded by neighboring genes located on locus 5q31.1 and share several epigenetic regulatory elements (3, 4). Both cytokines share the same receptor system and signal predominantly through the same transcription factor, namely: signal transducer and activator of transcription 6 (STAT6) (5). The shared signaling complex likely accounts for the fact that IL-13 was initially considered as an IL-4-like cytokine with redundant functions. However, over the past years it has become clearly apparent that the two cytokines possess several roles that are non-redundant and that STAT6-independent signaling also contributes to IL-4-induced functions, specifically in myeloid cells (6, 7). While early studies have focused on the roles of IL-4 and IL-13 in Th2 immune responses, our knowledge regarding the effector functions of IL-4 and IL-13 has substantially increased and it is now clear that IL-4 and IL-13 play roles that are
far beyond “classical” Th2 immunity (e.g. allergy and parasite infections) with functions in metabolism, tissue regeneration, remodeling, cancer and even learning and memory (8). This review will summarize the current knowledge regarding IL-4 and IL-13 with emphasis on their cellular source, signaling system and roles in allergy and beyond. In addition, we will outline current pharmacological strategies targeting these cytokines and/or their receptors.

1.1. The Cellular Source of IL-4 and IL-13

Many different cell types are capable of secreting IL-4 and IL-13 in-vitro. However, in-vivo, the cellular source for these cytokines is restricted to distinct tissues at specific time points (9). The development of new genetic tools, such as IL-4 and IL-13 reporter mice, which enabled the visualization of cytokine-expressing and/or producing cells under homeostatic conditions and in response to inflammatory triggers, enhanced our understating on the expression pattern of these cytokines in-vivo. As illustrated in Figure 1A, in response to antigen presentation and in the presence of IL-4 from a cellular source that is yet unclear, CD4+ Th2 cells can secrete both IL-4 and IL-13, either in a coordinated manner in which both cytokines are secreted from the same cell or in an un-coordinated manner in which a single cytokine is secreted from distinct Th2 cells, producing either IL-4 or IL-13 (10, 11) . Invariant NKT (iNKT) cells can secrete IL-4 (but not IL-13, Figure 1B) while group two innate lymphoid cells (ILC2) are the predominant source for IL-13 following exposure to alarmins such as IL-33 and to epithelial-derived cytokines such as IL-25 and TSLP (9, 12, 13)(Figure 1C). Eosinophils are a significant source for IL-4 during allergic responses and
helminth infections (9, 14, 15) (Figure 1B). Finally, basophils and mast cells can secrete IL-4 and IL-13 while T follicular helper (Tfh) cells have been shown to secrete IL-4 only (9, 15-19).

1.2. The IL-4 Receptor Signaling System

Although IL-4 and IL-13 share only 25% sequence identity, they have many overlapping functional properties (20-22). The basis for these overlapping functions stems from the fact that they share a common receptor subunit, and signaling pathway involving the Janus kinase (JAK):STAT6 pathway (23). The IL-4 receptor signaling system (Figure 2A) comprises two receptors, the type 1 IL-4 receptor (IL-4R) and the type 2 IL-4R (5, 24). The type 1 IL-4R is comprised of the IL-4Rα chain, which is paired with the common γ chain, while the type 2 IL-4R is comprised of the IL-4Rα chain paired with IL-13Rα1. IL-4 binds the IL-4Rα chain and therefore can signal both via the type 1 or type 2 IL-4Rs. In contrast, IL-13 binds IL-13Rα1 and can therefore signal via the type 2 IL-4R. Despite the fact that both IL-4 and IL-13 can utilize the type 2 IL-4R, they do so in a different manner (25). IL-4 binds IL-4Rα with high affinity (Kd~ sub-nanomolar) and the recruitment of either the common γ chain or IL-13Rα1 does not add to the affinity of the receptor. In fact, the recruitment of IL-13Rα1 to the pre-formed IL-4:IL-4Rα dimer is mediated by a low affinity interaction and is considered as a rate-limiting step. In contrast, IL-13 binds IL-13Rα1 with moderate affinity (Kd~ 30nM) but the recruitment of IL-4Rα to the pre-formed IL-13:IL-13Rα1 dimer increases the affinity of IL-13 to IL-13Rα1 to a picomolar level (25). Thus, IL-13 binding to IL-
13Rα1 favors the formation of the IL-13:IL-13Rα1:IL-4Rα receptor over the formation of IL-4:IL-13Rα1:IL-4Rα. An additional factor that contributes to the differential signals driven via the type 2 IL-4R by IL-4 and/or IL-13 is the relative abundance of each receptor chain on the cell surface. It is estimated that the number of IL-4Rα molecules on the cell surface is limited (~50-5000 molecules/cells). However, the number of IL-13Rα1 molecules on the surface of structural cells (e.g. epithelial cells, fibroblasts, endothelial cells, muscle cells) is relatively abundant (~5000-150000 molecules/cells) while its expression on B lymphocytes and monocytes is low and nearly absent on the surface of T lymphocytes (26-28). Collectively, unless IL-13Rα1 or the common γ chain are in substantial excess, only a portion of the IL-4:IL-4Rα complexes will achieve the capacity to signal. By contrast, the formation of the IL-13:IL-13Rα1:IL-4Rα complex is 25-fold more efficient than the formation of the IL-4:IL-13Rα1:IL-4Rα or the IL-4:γc:IL-4Rα complexes (25). Nonetheless, due to the lower affinity of IL-13 to IL-13Rα1, higher concentrations of IL-13 are needed in order to initiate signaling rather than IL-4 that will achieve maximum receptor signaling in much lower concentrations.

Cellular response to IL-4 or IL-13 via the type 1 or type 2 IL-4Rs are regulated also by the differential expression of the γc chain. Since the γc chain is predominantly expressed by immune cells, the type 1 IL-4R is widely expressed on immune cells of the lymphoid and myeloid lineage. In contrast, the type 2 IL-4R is widely expressed predominantly by non-hematopoietic cells such as epithelial cells and fibroblasts. Although the type 2 IL-4R is expressed by myeloid
cells it is almost completely absent on the surface of T and B cells (29). The differential utilization of the type 2 IL-4R along with the differential cellular expression of the type 1 and type 2 IL-4R, provides an elegant explanation for the functional differences between IL-4, which is considered as an immune-modulator cytokine and IL-13 that is considered as an effector cytokine. This also provides an explanation for some of the non-redundant functions of the two cytokines.

Adding complexity to this receptor signaling system, IL-13 can bind an additional receptor, termed IL-13Rα2 (30) (Figure 2C). IL-13Rα2 is expressed in two forms; a membrane-bound form (mIL-13Rα2) and a soluble form (sIL-13Rα2). mIL-13Rα2 and sIL-13Rα2 bind IL-13 with high affinity (20-90pM), which is ~100-300 fold higher than the binding of IL-13Rα1 to IL-13. The exact role of IL-13Rα2 in mediating IL-13 signaling is not fully understood. Due to the higher affinity of IL-13Rα2 in comparison with IL-13Rα1, the sIL-13Rα2 is considered as a decoy receptor, which can “capture” IL-13 and decrease the availability of this cytokine to bind IL-13Rα1. However, the soluble form of IL-13Rα2 is not expressed in humans (30). Thus, due to the lack of sIL-13Rα2 (and hence the lack of decoy receptor), it is more likely to assume that human IL-13Rα2 is capable of signaling. In support of this notion, human IL-13Rα2 was shown to signal in several human monocytic cell lines (31) and in glioblastoma multiforme (32).

1.2.1. Signal Transduction Following IL-4R Engagement
Initiation of signal transduction by the IL-4 receptors requires receptor-associated kinase phosphorylation since neither of the IL-4R chains contains endogenous kinase activity. The predominant signaling cascade initiated by IL-4R engagement is the Jak:STAT signaling pathway. IL-4Rα associates with Jak1 and Jak2, the γc chain associates with Jak3 and IL-13Rα1 can associate with Jak2 and Tyk2 (Figure 2A-B). Following engagement of IL-4 or IL-13 to their respective receptors, phosphorylation of Jak-kinases leads to the phosphorylation and dimerization of STAT6. Subsequently, STAT6 translocates into the nucleus and induces the transcription of IL-4/IL-13-target genes. Although STAT6 is the most prominent signaling pathway in IL-4 and/or IL-13-mediated responses, IL-4 can induce STAT6-independent signaling events via IL-4Rα as well. In fact, IL-4Rα can associate with insulin receptor substrate-1 and 2 (IRS1/2) that further recruits phosphoinositol-3 kinase (PI3K). IL-4-induced, PI3K-mediated signaling, was shown to regulate the survival of Th2 cells.

IL-4Rα is subjected to various levels of regulation. For example, biochemical studies have demonstrated that the IL-4Rα chain possesses an intrinsic immunoreceptor tyrosine-based inhibitory motif (ITIM), which can suppress IL-4 (and likely IL-13) signaling (33, 34). In addition, STIP1 homology and U-Box containing protein 1 (STUB1) interacts with IL-4Rα and targets it for degradation thus terminating IL-4 or IL-13 signaling (35) (Figure 2D). Furthermore, CD300f, is an Ig-superfamily receptor that mediates the engulfment of apoptotic cells by various myeloid cells in humans and mice. CD300f, at least in mice, has been...
shown to directly associate with IL-4Rα and co-amplify IL-4Rα-induced STAT6 signaling events in response to IL-4 and IL-13 (36) (Figure 2E).

2. Functions of IL-4 and IL-13 in Allergy and Beyond

Initially, the roles of the IL-4 and IL-13 emerged from the studies of parasitic infections. Indeed, the immune response to helminth parasites is generally considered as a dominant type 2 immune response which leads to worm expulsion. As investigations into the functions of IL-4 and IL-13 deepened, the role of these cytokines in the allergic responses was also established. Currently, it is appreciated that IL-4 and IL-13 have roles in multiple non-allergic, non-Th2 governed conditions (Figure 3). In the following sections, we will overview the roles of IL-4 and IL-13 in various disease settings and assess the potential therapeutic value of targeting IL-4 and/or IL-13 in these settings.

2.1 “Traditional” Functions of IL-4 and IL-13: Helminth Infections

Helminth parasites are multicellular, macro pathogens, which are the most common parasitic infections worldwide affecting ~24% of the world’s population (WHO, 2017). Infections are mostly distributed in tropical and sub-tropical areas and mainly in third world countries. Infections with helminth parasites typically result in a robust type 2 immune response, which is characterized by the secretion of IL-4, IL-13, IL-5 and IL-10 and the accumulation of effector cells such as eosinophils, basophils, alternatively activated macrophages and mast cells. In addition, it is accompanied production of IgG1, IgG4 and IgE antibodies
This host response is more accurately referred to as “modified-type 2” response since it usually encompasses high levels of IL-10 and low levels of IgE (39). From an evolutionary point of view, it seems that mammals mount a “tissue injury-like” response following recognition of invading helminths and that the host response to these parasitic infections is directed towards tolerance and resistance. A Th2-dominant response is quite universal in helminth infections (40), and in fact, certain aspects of the host response towards helminth infections are completely dependent on IL-4Rα and STAT6 signaling. Certainly, worm expulsion, a process that involves mucus secretion, muscle hypercontractility and accelerated epithelial cell turnover, is highly dependent on IL-4Rα function in most helminth infection models (41-44). However, while a type 2 immune response is considered protective in re-infection with several helminth parasites, the resistance in individuals that display natural immunity to infections, is predominantly mediated by a type 1 response (45). In addition, there are several examples demonstrating that early infection with Schistosomes and Brugia malayi promotes a type 1 immune response that is progressively replaced by a type 2 immune response (46, 47). Taken together, it is currently understood that distinct mechanisms are involved in the elimination of diverse helminth species and even more so of distinct life cycle stages of the same species.

2.1.1. IL-4 and IL-13 in Allergic Disease: The Instigator and the Effector Cytokine of the Allergic Response

The allergic response can be divided into two major phases: the sensitization phase and the effector phase. For example, in asthma sensitization to a specific
allergen occurs in atopic individuals long before the appearance of disease symptoms such as wheezing, bronchoconstriction and airway inflammation. During this asymptomatic stage, antigen presentation by dendritic cells will induce the differentiation of naïve CD4+ T cells into Th2 cells, differentiation of B cells into IgE secreting plasma cells and the binding of IgE antibodies to FcεR1 receptors on the surface of mast cells and basophils. IL-4 is critical for these initial responses and is therefore considered as a key instigator of the allergic response (48-50). Subsequently, following repeated exposure to the allergen, IgE cross-linking on mast cells may occur (in allergen-sensitized patients) causing the secretion of histamine, arachidonic acid metabolites (e.g. prostaglandins, leukotrienes) and various chemokines that induce the transmigration of inflammatory cells, most importantly eosinophils. This late-phase response is predominately regulated by IL-13 and characterized by the presence of eosinophils. Subsequently, increased IL-13 levels result in the manifestation of disease symptoms including epithelial cell hyperplasia, mucus hypersecretion and tissue remodeling (51-53), all of which can be directly mediated by IL-13. Accordingly, while many over-lapping functions for IL-4 and IL-13 are seen in the allergic response, IL-4 is generally considered more as the initiator of the allergic response and IL-13 as the effector cytokine responsible for clinically relevant disease symptoms.

2.1.2. Role in Asthma
The critical involvement of IL-4 and IL-13 in asthma onset and progression was clearly established early in the research of type 2 immune responses. Nonetheless, asthma is acknowledged as a heterogeneous disease encompassing several different phenotypes including eosinophilic asthma, neutrophilic asthma and mixed granulocytic asthma (54). A hallmark Th2-mediated response, which includes high eosinophilic count in the blood and bronchoalveolar (BAL) fluid and responsiveness to corticosteroids is currently referred to as eosinophilic asthma, which can be found in allergic and non-allergic individuals (55). This asthma endotype, is often associated with increased expression of IL-4 and IL-13 and therefore, targeting IL-4 and IL-13 during eosinophilic asthma could prove to be clinically significant (see Table 1 and section 3 below: “Therapeutic Targeting of the IL-4/IL-13 Pathway”).

2.1.3. Role in Atopic Dermatitis

Atopic Dermatitis (AD) is a chronic, relapsing, inflammatory disease of the skin. AD may precede the development of asthma and/or other allergic disorders in a phenomenon termed the “atopic march” (56), where skin sensitization in early childhood eventually may evolve into allergic reactions in other organs a such as the lung. Nonetheless, AD may occur without concomitant allergic sensitization and may not associate with an increased risk of asthma (57). The common features of AD are defective skin barrier, atopy, elevated Th2 response and predisposition to viral skin infections (58). AD patients are classified into two groups; extrinsic AD (approximately 80-90% of patients) manifests with skin inflammation accompanied by high IgE levels in the serum while intrinsic AD has
no allergen specific IgE or total IgE antibodies found in their blood or skin and is considered non-allergic AD (59). IL-13 expressing CD4\(^+\) Th2 cells and protein levels are higher in acute skin lesions than in chronic skin lesions. In general, IL-13 is expressed to a greater extent than IL-4 during disease progression (60). In addition, while high IL-13 levels and eosinophils counts are characteristic of extrinsic AD, lower IL-13 levels and eosinophil counts are found in intrinsic AD patients along with elevated Th17 and Th22 response (60, 61). Actually, IL-13 levels are directly correlated with disease severity and flares (62). Several studies assessing the roles of IL-4 and IL-13 in the etiology of AD highlighted their involvement in keratinocyte activation, leading them to secrete T cell chemoattractants. Furthermore, IL-4- and IL-13-activated keratinocytes displayed decreased barrier function. This is likely achieved by the ability of IL-4 and IL-13 to attenuate fibroblast and keratinocyte extracellular matrix proteins, such as matrix metalloproteinases (MMPs) and adhesion molecules expression (63). Taken together, neutralizing the activities of IL-4 and IL-13 in extrinsic AD patients might facilitate better disease management.

2.1.4. Role in Eosinophilic Esophagitis

EoE represents a chronic, local immune-mediated esophageal disease, characterized clinically by symptoms related to esophageal dysfunction and histologically by eosinophil-predominant inflammation. Importantly, systemic and/or local causes for esophageal eosinophilia should be excluded (64). Symptoms of EoE include dysphagia, food impaction, vomiting, pain and failure
to thrive (65). In accordance with the food allergen-driven nature of this disease, most patients will benefit greatly from dietary changes and antigen avoidance that can be achieved by elemental diet (66). However, while the majority of EoE patients will present IgE-mediated hypersensitivity to food allergens, non-IgE mediated reactions (delayed type, T cell mediated) are also recognized in the pathogenesis of EoE (67, 68). Thus, it is now appreciated that both allergen specific IgE-mediated and T-cell mediated allergic sensitization are independent mechanisms underlying the Th2 immune response in EoE. Key involvement for IL-13, TSLP and eotaxin-3 has been suggested in mediating the accumulation of eosinophils in EoE. For example, IL-13 is capable of inducing a remarkably similar epithelial cell transcriptome signature in mice (e.g. in experimental EoE) and human EoE patients. Furthermore, IL-13 is a potent inducer of eotaxin-3 in human esophageal epithelial cells, which is responsible for eosinophil accumulation in the esophagus (69, 70). Furthermore, IL-13 was shown to be involved in reduced barrier function, fibrosis, epithelial cell hyperplasia and angiogenesis during EoE (71-74). Taken together, anti-IL-13 therapy might be beneficial for the management of EoE. Nonetheless, decreasing IgE levels and/or Th2 sensitization by targeting IL-4 may be beneficial as well.

2.2. IL-13: The Two-Faced Cytokine in Tissue Remodeling

While tissue remodeling is an endpoint consequence of many different diseases stemming from multiple etiologies (e.g. parasitic infections, allergic disorders, cardiovascular disease, inflammatory bowel disease and cancer), in some diseases
the actual tissue damage, caused by fibrosis, is considered to be the disease itself. These diseases are considered “hallmark” fibrotic diseases. For example, two of these diseases that affect the lungs are chronic obstructive pulmonary disease (COPD) and idiopathic pulmonary fibrosis (IPF). While the pro-fibrogenic role of the Th2 cytokines, IL-4 and IL-13, is well established in parasitic infections and allergic diseases, the function of these cytokines in IPF seems to be more complex than previously appreciated.

IPF is an interstitial lung disease affecting 3-6 out of 100,000 people in the general population and is currently on the rise (75). IPF is characterized by a restrictive pattern of lung volume abnormality that is associated with impaired diffusion capacity following exposure to an idiopathic irritant (76). Although IPF is a relatively rare disease it is deadly with a median survival rate of less than three years (77). For many years it was accepted that chronic inflammation was the underlying cause of pulmonary fibrosis, nonetheless, the finding that IPF patients do not respond to glucocorticoid therapy challenged the contribution of ‘inflammation” per se, to IPF pathogenesis. The underlying pathology in IPF is apoptosis of epithelial cells, activation of fibroblasts and accumulation of extracellular matrix (ECM) (78), likely in response to repetitive micro injuries. Thus, it is currently hypothesized that during IPF, aberrant wound healing responses occurs in reaction to chronic stimuli in the lungs (78). IL-13 can regulate fibrosis in various ways, it can modulate fibroblast functions directly or through the regulation of TGF-β. In addition, IL-13 can promote synthesis of extracellular matrix proteins (ECM) and can promote alternative activation of macrophages, which are associated with fibrosis. Thus, IL-13 may
have a role in the pathogenesis of IPF (23, 79). Indeed, IL-13 levels were shown to be increased in IPF patients and fibroblasts isolated from IPF patients were hyper-responsive to IL-13 treatment (80, 81). However, the involvement of IL-13 in the pathogenesis of IPF remains controversial. IPF patients frequently present a type 1 pro-inflammatory cytokine profile rather than a type 2 cytokine profile. Furthermore, several studies report an IL-13 and IL-13Rα1 independent fibrosis in mouse models of pulmonary fibrosis. Finally, bleomycin-treated Il13ra1−/− mice displayed increased pathology likely due to a protective role for IL-13Rα1 in epithelial cells (82-84). Collectively, the involvement of IL-13 in the etiology of IPF and the possible targeting of this cytokine for therapy remain to be further investigated and tested by clinical trials.

3. Therapeutic Targeting of the IL-4/IL-13 Pathway

Over a dozen different therapeutic entities including; mutated ligands, soluble receptors, and antibodies have been developed in past years all aimed at targeting IL-4, IL-13 or IL-4Rα (Table 2). Based on in vitro and in vivo animal models, therapeutic targeting of IL-4/IL-13 held great promise. Nonetheless, early studies evaluating IL-4 and IL-13 targeting therapies showed disappointing results, mainly due to lack of efficacy. Importantly, dual IL-4/IL-13 neutralization by dupilumab, which showed promising results in clinical trials, has recently been approved by the FDA for the treatment of AD clinically validating the importance of this pathway in the pathophysiology of allergic diseases. In this section, we will review current and past therapeutics targeting the IL-4/IL-13 pathway.
3.1. **Entities Targeting IL-4**

Two entities were previously developed for targeting IL-4. Altrakincept (Immunex/Amgen) is a recombinant soluble IL-4Rα protein that inhibits IL-4 signaling by competing with the membrane receptor. Two phase I/II studies enrolling moderate asthmatics, either dependent or independent on inhaled corticosteroids (ICS) were conducted (85, 86). Interestingly, in these studies, Altrakincept was delivered via the inhalation route and had a serum half-life of approximately one week. In both trials, an improvement of lung functions (mainly FEV₁) was shown. However, no decrease in blood eosinophils or total serum IgE levels was observed and no additional studies were performed. A second entity neutralizing IL-4 (human IgG1, pascolizumab, GlaxoSmithKline) was under a phase II study on mild asthmatics. To date, the outcomes of this study were not reported (NCT00024544).

3.2. **Entities Targeting IL-13**

In agreement with the predominant effector functions of IL-13 in the effector stage of the allergic response, significantly more efforts were devoted to the therapeutic targeting of IL-13 (versus IL-4). Several different entities were tested clinically. Among those, studies using tralokinumab (MedImmune/AstraZeneca) and lebrikizumab (Genentech) were initially most promising.

Tralokinumab is a fully human anti-IL-13 IgG4 antibody that was tested in severe asthmatics, atopic dermatitis patients as well as IPF patients. Although Tralokinumab showed promising results in early phase II asthma trials (87, 88), but
according to topline results, which were announced by AstraZeneca and a summary of the STRATOS2 clinical trial (NCT02194699), failed to meet the primary end points of reduced annual exacerbations or reduction in the use of oral corticosteroids (OCS) in phase III studies.

Lebrikizumab, a fully humanized IgG4 mAb, also failed to improve clinical outcomes in phase II trials (89-94). Interestingly, in the two LAVOLTA phase III trials, despite meeting a statistically-significant reduction in exacerbations rate, which was the primary study end-point, the authors concluded that the overall effect was not clinically meaningful (95). Collectively, these data raise the question whether the selection of this primary end point was indeed indicative of efficacy and whether better stratification of patients was required.

The combined data emerging from strategies targeting a single cytokine and especially of IL-13 highlight the importance of IL-13 in asthma. Nonetheless, the data raise the notion that inhibition of IL-4 or IL-13 alone is not sufficient to manage clinically significant aspects of the disease. Thus, better strategies and biomarkers that will predict patient responsiveness to therapy should be developed.

3.3. Dual Targeting of IL-4/IL-13

Following inconsistent success with single cytokine targeting, significant advancements in the treatment of allergic diseases came from the dual targeting of IL-4 and IL-13. Four different drugs were developed so far for the targeting of both cytokines; a mutant IL-4 protein which antagonizes IL-4Rα (Pitrakinra, Aerovance),
antibody combination of anti-IL-4 and anti-IL-13 (QBX258, Novartis) and two anti-IL-4Rα antibodies (AMG-317, Amgen and dupilumab, Regeneron/Sanofi).

Dual cytokine targeting via dupilumab appears to be quite effective in allergic diseases and therefore dupilumab was rapidly termed an “asthma hit”. Dupilumab is a human IgG4 antibody targeting IL-4Rα, which has been primarily studied in the context of AD with great potency and was approved by the Food and Drug Administration (FDA) in March 2017. Nonetheless, dupilumab shows promising results in, asthma, chronic rhinosinusitis with nasal polyposis, and EoE. As of January 1st, 2018, dupilumab has been registered to more than 30 trials of which 17 are completed with relative success.

3.3.1. Dupilumab in Atopic Dermatitis

Four I/II phase combined studies, evaluated the efficacy and safety of dupilumab in moderate-to-severe atopic dermatitis (AD) patients using topical corticosteroids (TCS) and calcineurin inhibitors. The results of these studies were published simultaneously (96), demonstrating improvements in Eczema Area and Severity Index (EASI)-50, IGA, pruritus, and incidence of skin infections, while reducing TCS. Outcomes of these studies also suggested that clinical efficacy of dupilumab was independent of serum IgE levels as clinical benefits were observed at 4 weeks of treatment, whereas serum IgE levels were reduced only after 12 weeks. A subsequent phase IIb trial in moderate-to-severe inadequately controlled AD patients with topical medications, evaluated several dose regimens of dupilumab (300mg Q1W, 300mg Q2W, 300mg Q4W, 200mg Q2W, or 100mg Q4W). All dose
regimens significantly improved EASI, SCORing Atopic Dermatitis (SCORAD), and pruritus scores over placebo in a dose-dependent fashion (97). In addition, the phase III replicate studies SOLO 1 and SOLO 2 repeatedly showed the superiority of dupilumab over placebo with clinically significant improvements in IGA, EASI, pruritus, anxiety and depression (HADS), and quality of life (DLQI). Common adverse events in the dupilumab treated patients were injection-site reactions and conjunctivitis (98). An additional phase III study, CHRONOS, evaluated the efficacy and safety of dupilumab as an add-on therapy to TCS during a 52-week period. Dupilumab met the co-primary end points, IGA and EASI-75 by Week 16, and improvements in pruritus and SCORAD were observed as well, while reducing the use of TCS. These results were maintained for 52 weeks with comparable adverse events were reported in the dupilumab and placebo groups. Nonetheless, injection-site reactions and conjunctivitis were more common in patients treated with dupilumab (99). In March 2017, the FDA approved the use of dupilumab in adult atopic dermatitis patients whose disease is inadequately controlled with topical prescription medications. Dupilumab is also under evaluation for pediatric (aged 6-12) and adolescent (aged 12-18) AD patients. To date, one of these studies has been completed (NCT02407756) and the other one is still active (NCT03054428) while two additional studies involving patients under 18 years of age are recruiting (NCT02612454, NCT03345914). Finally, a phase I study is currently evaluating an auto-injector device in adults suffering from moderate-to-severe AD (NCT03050151).
3.3.2. Dupilumab in Asthma

Multiple phase II trials demonstrated the safety and efficacy of subcutaneous dupilumab in asthma. In a study of persistent eosinophilic asthma, subcutaneous dupilumab improved exacerbations rate by 87% and FEV\textsubscript{1} by 0.27L compared with placebo. These effects were sustained even after inhaled corticosteroids (ICS) and Long-Acting Beta Agonist (LABA) withdrawal. Moreover, dupilumab significantly reduced serum IgE, eotaxin, CCL17, and nitric oxide (NO) levels (100). A multinational study in an uncontrolled asthma population evaluated 4 dose regimens of dupilumab as an add-on therapy over 24 weeks. By Week 12, dupilumab improved FEV\textsubscript{1} and exacerbations rate in 3 of the dose regimens (200mg Q2W, 300mg Q2W, and 300mg Q4W)(101). Another study is ongoing to evaluate the effect of dupilumab on airway inflammation in persistent asthma (NCT02573233). Topline results from two phase III studies have been released by Regeneron & Sanofi. In the QUEST study on persistent asthma patients (NCT02414854), dupilumab met the two primary end points, FEV\textsubscript{1} (0.13L increase) and exacerbations rate (46% decrease) over placebo in the overall population. Patients with medium or high eosinophil counts showed greater benefits with 0.24L increase in FEV\textsubscript{1} and 67% decrease in exacerbations rate in the eosinophil-high subgroup. In the VENTURE study (NCT02528214), dupilumab was evaluated as an add-on therapy to the standard of care in severe, oral corticosteroids (OCS)-dependent asthma. At 24 weeks, dupilumab reduced the use of OCS in the overall population, and, despite reducing OCS, exacerbations rate was reduced by 59% and FEV\textsubscript{1} was increased by 0.22L compared with placebo. In eosinophil-high patients, dupilumab decreased
exacerbations rate by 71% and increased FEV\(_1\) by 0.32L. Most impressively, half of dupilumab-treated patients have completely eliminated their use of OCS. Full publication of peer-reviewed data from QUEST and VENTURE is eagerly awaited. The long-term efficacy and safety of dupilumab in pediatric patients is currently being assessed (NCT02948959).

3.3.3. *Dupilumab in chronic rhinosinusitis and nasal polyps*

In a single phase II study that was conducted in chronic rhinosinusitis and nasal polyposis (CSwNP) patients, dupilumab was evaluated as an add-on therapy to mometasone furoate nasal spray. Dupilumab met the primary end point of improved nasal polyp score. Notably, larger benefits were observed in patients with co-morbid asthma, a subset of which also showed FEV\(_1\) and Asthma Control Questioner (ACQ) improvement. Common side effects included nasopharyngitis, injection-site reactions, and headaches (102). Two phase III studies, SINUS-24 and SINUS-52 are ongoing to evaluate dupilumab as an add-on therapy in CSwNP.

3.3.4. *Additional entities targeting IL-4R\(\alpha\)*

Pitrakinra and AMG-317 represent additional and distinct therapeutic entities targeting IL-4R\(\alpha\). Pitrakinra is a recombinant mutated IL-4 variant that binds IL-4R\(\alpha\) and prevents its complexing with \(\gamma_c\) and IL-13R\(\alpha_1\) chains, thereby neutralizes signaling of both the type 1 and type 2 IL-4Rs (103). AMG-317, a monoclonal antibody to IL-4R, was tested in a phase II trial using several doses in moderate-to-
severe asthmatics with disappointing results (104). This trial tested 75, 150, or 300mg subcutaneous injection of AMG-317 compared with placebo in moderate-to-severe asthma. None of the dose regimens showed improvement of ACQ, which was the primary end point, or key secondary end points in the overall population. Nonetheless, a pre-planned analysis revealed clinically significant improvements in patients displaying the highest disease severity (105). These results are surprising especially in light of dupilumab's success to meet similar clinical endpoints. A plausible explanation for the different activities of these drugs may be due to the high clearance of AMG-317. A following PK analysis (105) identified a high clearance rate for AMG-317 of 35.0 mL/hr, which is, for instance, ~3 times higher than tralokinumab's clearance rate (8mL/hr)(106). Therefore, AMG-317 may have not sufficiently blocked IL-4 and IL-13 in this trial. This hypothesis was not assessed during the trial.

3.3.5. Dual IL-4/IL-13 targeting: Future perspectives

To date, the majority of patients that were recruited for clinical trials assessing therapeutics targeting IL-4/IL-13, were selected based on peripheral blood eosinophil counts and serum IgE levels. Given that complex disease phenotypes are observed in patients (presence or lack of IgE secretion, elevation or no change in eosinophil numbers, dependency or independency on corticosteroids and more) as well as the distinct asthma endotypes which have been characterized, it is reasonable to assume that better patient stratification according to the inflammatory cell composition, selected biomarkers and resistance to
corticosteroids would enable “tailored” treatment that might yield better clinical outcomes than those observed thus far. Moreover, the clinical benefit, which was observed in patients that have been treated with dupilumab raise the notion that dual targeting of IL-4 and IL-13 signaling may be superior to single cytokine strategies by targeting IL-4 or IL-13. It would be interesting to assess whether targeting IL-13Rα1, which can block IL-4 and IL-13 binding and subsequent signaling via the type 2 IL-4 receptor, would also prove beneficial.
4. **New Roles for IL-4 and IL-13: From the Bone to the Brain**

Owing to the development of new mouse models and technologies, that enable to assess the roles of IL-4, IL-13 and their receptor chains, it has become increasingly apparent that IL-4 and IL-13 have additional activities, which are not restricted to allergy and parasitic infections.

4.1.1. **IL-4 and IL-13 in Metabolism**

The structure and homeostasis of the adipose tissue is tightly regulated by the immune system. Under homeostatic conditions, the lean white adipose tissue is associated with an anti-inflammatory Th2-oriented environment. It is mainly populated with alternatively-activated macrophages, eosinophils, ILC2 cells and T regulatory cells. Furthermore, the cytokine milieu associated with the lean tissue is characterized by TGF-β, IL-10, IL-4 and IL-13 (107, 108). Obesity is a risk factor for numerous metabolic diseases that are collectively termed the metabolic syndrome. These diseases include type 2 diabetes, non-alcoholic liver disease, heart disease and cancer (109). Interestingly, low-grade chronic inflammation is an underlying pathology of obesity-associated metabolic syndrome. Under obese-conditions, the adipose tissue switches from an anti-inflammatory environment, to a pro-inflammatory one (110, 111) where the expression of TNF-α, IL-1β, IL-6 and IL-8 (all of which have been implicated in mediating insulin resistance) are increased (111, 112). It appears eosinophil-macrophage cross-talk is a major axis in glucose metabolism and insulin resistance. Recently, a new paradigm has emerged where in the adipose tissue eosinophils provide IL-4 to sustain the alternatively activated state of resident
macrophages which secrete catecholamines to promote metabolic homeostasis (111, 113). Consequently, eosinophil-derived IL-4 is in the center of an immune-metabolic axis that forestalls obesity and glucose tolerance by promoting energy expenditure or by mechanisms yet to be defined (114). Upon increased caloric intake, eosinophils and subsequently eosinophil-derived IL-4 levels are decreased in the adipose tissue. Consequently, the adipose tissue is inflamed and becomes prone to metabolic disorders (113, 114). Thus, a tight balance between a Th1 and a Th2 environment is necessary for maintaining the homeostasis of the adipose tissue and to avoid adipose tissue fattening. Finally, IL-13 was shown to directly regulated glucose metabolism in the liver, and type 2 diabetics were shown to have lower IL-13 levels in the serum and skeletal muscle cells (115, 116). Taken together, it is possible that enhancing “protective” signaling by IL-4 and/or IL-13 could be used to control glucose levels and to treat type 2 diabetes. Nonetheless, strategies aimed at increasing IL-4 and/or IL-13 signaling should be done with caution due to the co-morbidities that are associated with metabolic diseases. For example, obese asthma patients exhibit increased release of leptin that primes and enhances eosinophil responses towards eotaxin (117). Thus, enhancing IL-4 and/or IL-13 signaling, which increase chemokine levels (118), may actually favor the migration and presence of eosinophils into the lungs and thereby promote a deleterious effect.

4.1.2. Th2 Cytokines in Bone Resorption
The bone is a dynamic tissue, where bone formation and bone resorption processes are tightly controlled to preserve skeletal size, shape, and structural integrity as well as mineral homeostasis (119). Bone resorption is an important process, which if un-regulated, can result in significant pathologies such as osteoporosis (120). Osteoclasts are myeloid cells of the monocyte/macrophage lineage that are exclusively responsible for bone resorption. In past years, it has become clear that IL-4 is an important regulator of bone resorption. IL-4 can directly suppress the differentiation of bone-marrow precursors to osteoclasts, and can suppress bone resorption by osteoclasts (121-123). Indeed, IL-4 levels were found to be lower in postmenopausal women with low bone mineral density than in women with normal bone mineral density and in osteopenic women (124). Thus, enhancing IL-4 levels could help treat osteoporosis by reducing osteoclastogenesis and bone resorption. However, strategies enhancing IL-4 and/or IL-13 signaling should be carefully examined due to possible unwarranted side effects such as systemic or local eosinophilia.

4.1.3. The Role of Th2 Cytokines in Cognitive Brain Functions

The brain is generally perceived to be shielded from immune cells. Yet, the importance of immune cells, mainly T cells, in the maintenance of brain integrity has been recently described. Cognitive decline, accompanying ageing and neurodegenerative diseases such as Alzheimer’s disease and multiple sclerosis, are associated with an increase in inflammation. This inflammatory state is skewed towards a Th1 phenotype with increased expression of IL-6, IL-1β and IL-18.
especially in the hippocampus (125). In contrast, the effect of a Th2-skewed inflammation on cognitive functions has been found to be protective. Meningeal Th2 cell numbers are increased following visuospatial learning and memory tasks as measured using a Morris water maze (MWM) test (126). IL-4 and IL-13, which are secreted from these Th2 cells, are able to stimulate astrocytes to produce brain-derived neurotrophic factor (BDNF) (126, 127). T cell deficient mice, \textit{Il4} deficient mice and \textit{Il13} deficient mice display severe cognitive impairment in learning tasks in the MWM test (126-128). In addition, IL-13Ra1 expression on dopaminergic neurons was shown increase their susceptibility to oxidative stress-mediated damage thereby contributing to their preferential loss in Parkinson’s disease. Interestingly, the \textit{Il13ra1} gene lies on the X chromosome within the PARK12 locus of susceptibility to Parkinson’s disease (129). Taken together, these studies establish an intriguing new role for IL-4 and IL-13 during learning and memory and highlight the possible involvement of these cytokines during neurodegenerative diseases.

5. \textit{Summary}

IL-4 and IL-13 are hallmark Th2-immunity regulating cytokines with fundamental roles in the initiation and progression of several diseases, some of which are “hallmark” Th2-asspociated diseases while others far from being “classical” Th2-immune disorders. These two cytokines show complex, pleotropic functions in disease pathophysiology. While intensive research has established the importance of both IL-4 and IL-13 in the etiology of the allergic response,
therapeutic targeting of the IL-4/IL-13 pathway has proven less productive than expected. Explanations to this conundrum could come from differences between mouse models and human diseases, from patient classification and from the lack of monitoring mechanistic aspects of treatment in clinical trials. For example, the levels of IL-4 and/or IL-13 and/or IL-4R chains are almost never assessed in patients before, during, or after treatment with anti-IL-4/IL-13 therapy. Sub-classification of patients according to cytokine or receptor levels along with IgE levels, eosinophil counts and additional biomarkers could help the interpretation of clinical results and enable a patient-tailored treatment.

Finally, the clear involvement of the IL-4/IL-13 pathway in newly appreciated conditions highlights the possible therapeutic potential of this pathway in non-allergic disease settings and could prove fruitful in the future. Importantly, while treatment of Th2-associated diseases such asthma, EoE and AD would require the inhibition/neutralization of IL-4 and/or IL-13 function, treatment of other diseases such as osteoporosis, diabetes or neurodegenerative diseases might require the enhancement of IL-4 and/or IL-13 activities, depending on the specific function of the pathway in disease pathophysiology.
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**Conflict of interest declaration**

Ariel Munitz is scientific consultant for GlaxoSmithKline, Compugen and Augmanity Nano LTD. DKA is an employee of Augmanity Nano LTD. IB and AB declare no conflict of interest.
References


Figure legends

Figure 1. Cellular sources for IL-4 and IL-13 in-vivo

Activation of T follicular helper cells (Tfh) in secondary lymphoid organs with antigen (in the presence of IL-4 from a yet unknown cellular source) induces them to produce IL-4 (A). Tfh-derived IL-4 is critical for initiating B cell-dependent humoral responses. During Th2 immune responses, naïve T helper cells, in secondary lymphoid organs and/or peripheral tissues, will be differentiated into a Th2 state following antigen presentation by antigen-presenting cells (mainly dendritic cells) in the presence of IL-4. This antigen-dependent stimulation is responsible for secretion of significant amounts of IL-4 and IL-13 establishing Th2 cells a major source for both cytokines (A). In peripheral tissues, diverse Th2-associated stimuli including IL-4, IL-13, IL-3, IL-5, alarmins (e.g. IL-33) and epithelial cell-derived cytokines (e.g. IL-25 and TSLP) can stimulate IL-4 secretion (but not IL-13) from iNKT cells, eosinophils and mouse basophils (human basophils were reported to also secrete IL-13) (B). Mast cells can secrete IL-4 and IL-13 following IgE cross-linking or following stimulation with IL-33 (B). ILC2 cells are a significant source for IL-5, an eosinophil priming and activation factor, which contributes to eosinophil-derived IL-4 secretion (C). ILC2 cells are also a major source for IL-13 following stimulation with alarmins and especially with IL-33 (C). Collectively, the induction of an IL-4/IL-13 Th2 response regulates the allergic immune-response (including mucus production, smooth muscle contraction and airway hyper-responsiveness) and supports anti-helminth immunity.

IL- Interleukin; Th2- T helper type 2; TSLP- Thymic stromal lymphopoenetin
Figure 2. The IL-4 receptor system

IL-4 and IL-13 signal via a complex network of receptors (R). These receptors comprise of several receptor chains, which upon ligand binding dimerize and form the type 1 IL-4R and the type 2 IL-4R (A). The type 1 IL-4R comprises the IL-4Rα chain along with the common γ chain (γc). The type 2 IL-4R (B) comprises the IL-4Rα chain along with the IL-13Rα1 chain. IL-4 binds with high affinity IL-4Rα and therefore can signal via either the type 1 or type 2 IL-4R’s. In contrast, IL-13 binds IL-13Rα1 and can therefore signal only via the type 2 IL-4R. In addition, IL-13 can also bind IL-13Rα2 with high affinity. IL-13Rα2 functions as a decoy receptor for IL-13 signaling in mice due to its availability in soluble form (C). However, in humans IL-13Rα2 is expressed in a membrane bound form and can this elicit potential signaling via ERK-1/AP-1 dependent pathways(C). Following ligand binding to either the type 1 or type 2 IL-4R’s the tyrosine phosphorylation of Irs1/2 and Jak1 occurs. In addition, the type 1 IL-4R can recruit Jak3 and the type 2 IL-4R can recruit Jak2 and Tyk2. Finally, the phosphorylation and activation of signal transducer and activator of transcription 6 (STAT6) occurs and subsequently IL-4/IL-13-dependent genes are transcribed. In addition, following IRS1/2 phosphorylation, PI3K can be recruited and phosphorylated, enabling STAT-6-independent signaling. IL-4Rα signaling may be regulated by various know mechanisms; 1) IL-4Rα contains an intracellular intrinsic ITIM domain, which can suppress IL-4Rα signaling by recruiting phosphatases; 2) Recruitment of the chaperon STUB1 (D) marks IL-4Rα for
degradation; and 3) CD300f, an Ig-superfamily receptor, can physically associate with IL-4Rα (at least in mice) and acts to amplify IL-4 and IL-13-induced effects via the type 1 and type 1 IL-4R’s (E). IRS1/2- Insulin receptor substrate, JAK-Janus kinase; STUB1- STIP1 Homology And U- Box Containing Protein 1; TYK-tyrosine kinase, SHP- Src homology region 2 domain-containing phosphatase-; sIL-13Rα2- soluble IL-13Rα2; mIL-13Rα2- membrane IL-13Rα2, PI3K-Phosphatidylinositol-4,5-bisphosphate 3-kinase; STAT6- Signal transducer and activator of transcription 6.
Figure 3. Pleotropic function of IL-4 and IL-13

IL-4 and IL-13 play distinct yet pleotropic functions in various diseases. While helminth infections are considered as hallmark Th2-associated diseases, it is becoming apparent that Th1 cytokines are involved in early infection events. In asthma IL-4 is responsible for the initiation of the allergic response and IL-13 for later chronic events leading to tissue remodeling and fibrosis. IL-13 is the main regulator of eosinophilic esophagitis and is also considered as a main pro-fibrotic factor in idiopathic pulmonary fibrosis (IPF). In contrast, the process of bone resorption is mainly regulated by IL-4 and both IL-4 and IL-13 are key regulators of atopic dermatitis, adipose tissue homeostasis and glucose metabolism and brain cognitive functions such as learning and memory.
**Table 1: Predicted outcomes of therapies targeting IL-4, IL-13 and their receptors: asthma as an example**

<table>
<thead>
<tr>
<th>Predicted benefit</th>
<th>Anti-IL-4</th>
<th>Anti-IL-13</th>
<th>Anti-IL-4Rα</th>
<th>Anti-IL-13Rα1</th>
</tr>
</thead>
<tbody>
<tr>
<td>Th2 cytokine levels</td>
<td>↓</td>
<td>--</td>
<td>↓</td>
<td>--</td>
</tr>
<tr>
<td>Smooth muscle contraction</td>
<td>↓</td>
<td>↓</td>
<td>↓</td>
<td>↓</td>
</tr>
<tr>
<td>Antibody production (IgE/IgG)</td>
<td>↓</td>
<td>--</td>
<td>↓</td>
<td>--</td>
</tr>
<tr>
<td><strong>Effects on epithelial cells</strong></td>
<td>↓ (to some extent)</td>
<td>↓</td>
<td>↓</td>
<td>↓</td>
</tr>
<tr>
<td>(Mucus production)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tissue remodeling</td>
<td>↓ (to some extent)</td>
<td>↓</td>
<td>↓</td>
<td>↓</td>
</tr>
<tr>
<td>(Subepithelial and parenchymal fibrosis)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Inflammatory cell recruitment</td>
<td>↓</td>
<td>↓ (to some extent)</td>
<td>↓</td>
<td>↓ (to some extent)</td>
</tr>
<tr>
<td>Drug</td>
<td>Patient population</td>
<td>Patient numbers</td>
<td>Phase and study period</td>
<td>Dose</td>
</tr>
<tr>
<td>-----------------------------</td>
<td>----------------------------------</td>
<td>-----------------</td>
<td>------------------------</td>
<td>-------------------------------------------</td>
</tr>
<tr>
<td><strong>Targeting IL-4</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Altrakincept&lt;sup&gt;1&lt;/sup&gt;</td>
<td>Moderate atopic asthma</td>
<td>25</td>
<td>Phase I/II 29 days</td>
<td>0.5 or 1.5mg nebulized single dose</td>
</tr>
<tr>
<td></td>
<td>Moderate persistent asthma</td>
<td>62</td>
<td>Phase II 12 weeks</td>
<td>0.75, 1.5, or 3mg nebulized weekly</td>
</tr>
<tr>
<td></td>
<td>Asthma</td>
<td>60 per group</td>
<td>Phase III 12 weeks</td>
<td>0.9, or 1.8mg nebulized weekly</td>
</tr>
<tr>
<td>Pascolizumab&lt;sup&gt;2&lt;/sup&gt; Humanized IgG1</td>
<td>Mild asthma</td>
<td>120</td>
<td>Phase II Unreported</td>
<td>Unreported</td>
</tr>
<tr>
<td></td>
<td>Pulmonary Tuberculosis</td>
<td>32</td>
<td>Phase II 24 weeks</td>
<td>0.05-10mg/kg IV</td>
</tr>
<tr>
<td><strong>Targeting IL-13</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tralokinumab Human IgG4</td>
<td>Uncontrolled asthma</td>
<td>194</td>
<td>Phase Ila 13-weeks</td>
<td>150, 300, or 600mg SC Q2W</td>
</tr>
<tr>
<td></td>
<td>Severe asthma</td>
<td>452 (98 sites)</td>
<td>Phase IIb 52 weeks</td>
<td>300mg SC Q2W or Q2/4W</td>
</tr>
<tr>
<td></td>
<td>Asthma inadequately controlled on ICS</td>
<td>79</td>
<td>MESOS Phase II 12 weeks</td>
<td>300mg SC Q2W</td>
</tr>
<tr>
<td></td>
<td>Uncontrolled asthma</td>
<td>1207</td>
<td>STRATOS 1 Phase III 52 weeks</td>
<td>300mg SC Q2W or Q4W</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>STRATOS 2 Phase III 52 weeks</td>
<td>300mg SC Q2W</td>
</tr>
<tr>
<td></td>
<td>OCS-dependent asthma</td>
<td>140</td>
<td>TROPOS Phase III 40 weeks</td>
<td>300mg SC Q2W</td>
</tr>
<tr>
<td></td>
<td>Moderate-severe atopic dermatitis</td>
<td>204</td>
<td>Phase II 3 doses (unreported) SC Q2W</td>
<td>Significant dose-dependent reduction in EASI but not in IGA (p = 0.063) (primary end points).</td>
</tr>
<tr>
<td>Lebrikizumab&lt;sup&gt;III&lt;/sup&gt; Humanized IgG4</td>
<td>780</td>
<td>ECZTRA 1 Phase III 52 weeks</td>
<td>Unreported</td>
<td>Recruiting</td>
</tr>
<tr>
<td>--------------------------------------</td>
<td>-----</td>
<td>-----------------------------</td>
<td>------------</td>
<td>------------</td>
</tr>
<tr>
<td>780</td>
<td>ECZTRA 2 Phase III 52 weeks</td>
<td>Unreported</td>
<td>Recruiting</td>
<td>NCT03160885</td>
</tr>
<tr>
<td>Mild-moderate idiopathic pulmonary fibrosis</td>
<td>176</td>
<td>Phase II 68 weeks</td>
<td>400 or 800mg IV Q4W</td>
<td>Terminated early due to lack of efficacy</td>
</tr>
<tr>
<td>Mild allergic asthma</td>
<td>29</td>
<td>Phase II 12 weeks</td>
<td>5mg/kg SC Q4W</td>
<td>Statistically insignificant 48% reduction in LAR (primary end point). Significant reductions of serum IgE, CCL13, and CCL17. The drug was well tolerated.</td>
</tr>
<tr>
<td>Inadequately controlled on ICS</td>
<td>219</td>
<td>Phase II 24 weeks</td>
<td>250mg SC monthly</td>
<td>Significant FEV&lt;sub&gt;1&lt;/sub&gt; improvement (primary end point) and reductions of serum IgE, CCL13, and CCL17. Patients with elevated periostin levels achieved greater improvements. No drug-related adverse events were observed.</td>
</tr>
<tr>
<td>Steroid-naïve asthma</td>
<td>212</td>
<td>Phase II 12 weeks</td>
<td>125, 250, or 500mg SC monthly</td>
<td>There were no improvements in FEV&lt;sub&gt;1&lt;/sub&gt; (primary end point) in the overall population or in the periostin-high subgroup.</td>
</tr>
<tr>
<td>Mild-moderate asthma without ICS</td>
<td>310</td>
<td>Phase II 12 weeks</td>
<td>125mg SC Q4W</td>
<td>83ml increase in FEV&lt;sub&gt;1&lt;/sub&gt; (p = 0.6) (primary end point). The drug was safe and well tolerated.</td>
</tr>
<tr>
<td>Severe uncontrolled asthma</td>
<td>463</td>
<td>LUTE &amp; VERSE Phase II replicates</td>
<td>37.5, 125, or 250mg SC Q4W</td>
<td>Significant reductions in exacerbations rate (primary end point) without dose-response. Periostin-high patients had greater FEV&lt;sub&gt;1&lt;/sub&gt; improvements. The drug was well tolerated.</td>
</tr>
<tr>
<td>Uncontrolled asthma</td>
<td>1081</td>
<td>LAVOLTA 1 Phase III 52 weeks</td>
<td>37.5 or 125mg SC Q4W</td>
<td>Reduction in exacerbations rate in periostin-high patients (primary end point) was statistically-significant, but not clinically meaningful.</td>
</tr>
<tr>
<td></td>
<td>1067</td>
<td>LAVOLTA 2 Phase III 52 weeks</td>
<td></td>
<td></td>
</tr>
<tr>
<td>OCS-dependent asthma</td>
<td>230</td>
<td>Phase II 44 / 76 weeks</td>
<td>3 doses (unreported) SC Q4W</td>
<td>Completed (awaiting results)</td>
</tr>
<tr>
<td>IMA-026&lt;sup&gt;IV&lt;/sup&gt; Humanized IgG1</td>
<td>Mild atopic asthma</td>
<td>56</td>
<td>Phase II 35 days</td>
<td>2mg/kg SC on Days 1 and 8</td>
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<tr>
<td>Anrukizumab&lt;sup&gt;II&lt;/sup&gt; (IMA-638) Humanized IgG1</td>
<td>Persistent asthma</td>
<td>65</td>
<td>Phase II 112 days</td>
<td>0.2, 0.6, or 2mg/kg SC on Days 1, 8, 28, 56, and 84</td>
</tr>
<tr>
<td><strong>GSK679586</strong>&lt;sup&gt;ii&lt;/sup&gt; Humanized IgG1</td>
<td>Mild asthma</td>
<td>28</td>
<td>Phase I 8 weeks</td>
<td>2.5, 10, 20mg/kg IV monthly</td>
</tr>
<tr>
<td>Severe asthma</td>
<td>198</td>
<td>Phase II 12 weeks</td>
<td>10mg/kg IV monthly</td>
<td>No improvement in ACQ (primary end point), FEV&lt;sub&gt;1&lt;/sub&gt;, or exacerbations rate. No drug-related adverse events were observed. (132)</td>
</tr>
<tr>
<td>Dectrekumab&lt;sup&gt;ii&lt;/sup&gt; (QAX576) Unreported</td>
<td>Persistent asthma</td>
<td>259</td>
<td>Phase II 24 weeks</td>
<td>6mg/kg IV Q3W</td>
</tr>
<tr>
<td></td>
<td>PPI-resistant eosinophilic esophagitis</td>
<td>23</td>
<td>Phase II 12 weeks</td>
<td>6mg/kg IV Q4W</td>
</tr>
</tbody>
</table>

**Targeting IL-4 and IL-13**

<p>| <strong>Pitrakinra</strong>&lt;sup&gt;ii,v&lt;/sup&gt; Mutant IL-4 (IL-4Rα antagonist) | Mild asthma | 24 | Phase Ila 29 days | 25mg SC daily | No improvement in FEV&lt;sub&gt;1&lt;/sub&gt; (primary end point). The drug was safe. (134) |
| | | 32 | Phase Ila 28 days | 60mg nebulized twice daily | Significant improvement in FEV&lt;sub&gt;1&lt;/sub&gt; (primary end point). Asthma-related adverse events were not assessed. NCT00801853 |
| | Uncontrolled asthma | 534 | Phase II 12 weeks | 1, 3, or 10mg nebulized | No effect on the overall population. However, in eosinophil-high patients, exacerbation rate (primary end point) and symptom scores were significantly reduced. The safety profile was satisfactory. |
| | Moderate-severe atopic dermatitis | 25 | Phase II 1 month | 30mg SC twice a day | Significant reduction in disease flares (65%) and total IgE (25%), but only trends towards improvement in EASI. (63) NCT00676884 |
| <strong>QBX258</strong>&lt;sup&gt;v&lt;/sup&gt; Combination of IL-13 (QAX576) and IL-4 (VAK694) antibodies | Moderate-severe asthma | 65 | Phase II 12 weeks | QAX576: 6mg/kg VAK694: 3mg/kg IV infusion Q4W | Significant but minor reduction (0.514) in ACQ-7 (primary end point), without FEV&lt;sub&gt;1&lt;/sub&gt; improvement. NCT01479595 |
| <strong>AMG-317</strong>&lt;sup&gt;ii&lt;/sup&gt; Human IgG2 (IL-4Rα antagonist) | Moderate-severe asthma | 294 | Phase II 12 weeks | 75, 150, or 300mg SC weekly | No improvements in the overall population. Patients with higher baseline ACQ scores (&gt;2.86) were more likely to respond. The drug was safe and well tolerated. (104) |
| <strong>Dupilumab</strong> Human IgG4 (IL-4Rα antagonist) | Persistent eosinophilic asthma | 104 | Phase II 12 weeks | 300mg SC weekly | 87% reduction in exacerbations rate (primary end point) and FEV&lt;sub&gt;1&lt;/sub&gt; improvement. Significant reductions of biomarker levels were observed: serum IgE, eotaxin, TARC, and NO. Drug-related adverse events included injection-site reactions, nasopharyngitis, nausea, and headache. (100) |
| | Uncontrolled persistent asthma | 769 (174 sites) | Phase IIb 24 weeks | 200 or 300mg SC Q2W or QW4 | Dupilumab significantly improved FEV&lt;sub&gt;1&lt;/sub&gt; (primary end point) and exacerbations rate in all dose regimens, irrespective of eosinophil counts. Drug-related adverse events included upper respiratory tract infections, and injection-site reactions. (101) |
| | Persistent asthma | 1902 (413 sites) | QUEST Phase III | 200 or 300mg SC Q2W | Completed (awaiting results) NCT02414854 |</p>
<table>
<thead>
<tr>
<th>Study Type</th>
<th>Study Name</th>
<th>Duration</th>
<th>Treatment Details</th>
<th>Results</th>
<th>Notes</th>
</tr>
</thead>
<tbody>
<tr>
<td>OCS-dependent asthma</td>
<td>VENTURE</td>
<td>52 weeks</td>
<td>300mg SC Q2W</td>
<td>Press release: dupilumab reduced exacerbations and improved lung function in both dose regimens, with a greater effect in patients with elevated eosinophil counts.</td>
<td>Regeneron's press release on 11 Sep 2017 and Sanofi's press release on 31 Oct 2017</td>
</tr>
<tr>
<td>Pediatric uncontrolled persistent asthma</td>
<td>VOYAGE</td>
<td>52 weeks</td>
<td>SC Q2W</td>
<td>Significant dose-dependent improvements in EASI, IGA, pruritus, skin infections, and reductions of biomarker levels and TCS use. Drug-related adverse events included nasopharyngitis and headache.</td>
<td>NCT02948959</td>
</tr>
<tr>
<td>Moderate-severe atopic dermatitis</td>
<td>Four trials</td>
<td>16 weeks</td>
<td>75, 150, or 300mg SC weekly</td>
<td>Significant improvements in EASI, IGA, pruritus, skin infections, and reductions of biomarker levels and TCS use. Drug-related adverse events included nasopharyngitis and headache.</td>
<td>(96)</td>
</tr>
<tr>
<td></td>
<td>SOLO 1</td>
<td>16 weeks</td>
<td>300mg SC Q1W or Q2W</td>
<td>Significant improvements in IGA (primary end point), EASI, pruritus NRS, DLQI, and HADS, in both dose regimens. Injection-site reactions and conjunctivitis were more frequent in the dupilumab groups</td>
<td>(97)</td>
</tr>
<tr>
<td></td>
<td>SOLO 2</td>
<td>16 weeks</td>
<td></td>
<td></td>
<td>(98)</td>
</tr>
<tr>
<td>Moderate-severe atopic dermatitis on concomitant TCS</td>
<td>CHRONOS</td>
<td>52 weeks</td>
<td>300mg SC Q1W or Q2W</td>
<td>Significant improvements in IGA, EASI (co-primary end points), and pruritus at Weeks 16 and 52, while reducing TCS use. Drug-related adverse events included Injection-site reactions and conjunctivitis.</td>
<td>(99)</td>
</tr>
<tr>
<td>Pediatric and adolescent atopic dermatitis</td>
<td>Phase II</td>
<td>Unreported</td>
<td></td>
<td>4 trials evaluating the safety and efficacy of dupilumab in patients aged 6-18.</td>
<td>NCT02407756 and NCT02612454 and NCT03054428 and NCT03345914</td>
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<tr>
<td>Chronic sinusitis and nasal polyposis</td>
<td>Phase II</td>
<td>16 weeks</td>
<td>600mg loading + 300mg SC weekly</td>
<td>Improved nasal polyp score (primary end point) and secondary endpoints when added to standard of care (mometasone furoate) when compared to mometasone furoate alone. Drug-related adverse events included nasopharyngitis, injection site reactions, and headache.</td>
<td>NCT02912468</td>
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<tr>
<td>Condition</td>
<td>Phase</td>
<td>Duration</td>
<td>Treatment</td>
<td>Notes</td>
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<td></td>
</tr>
<tr>
<td>Active eosinophilic esophagitis</td>
<td>Phase II</td>
<td>12 weeks</td>
<td>300mg SC weekly</td>
<td>Completed (awaiting results) Press release: 26% improvement in primary end point (SDI – ability to swallow), 41% improvement in EoE-ERFS, and a 107% reduction in overall peak intraepithelial eosinophil count from baseline.</td>
<td></td>
</tr>
<tr>
<td>SINUS-52 Phase III 52 weeks</td>
<td>360</td>
<td>SC Q2W or Q2W until week 24 and then Q4W</td>
<td>Ongoing: to evaluate the efficacy of dupilumab in reducing nasal congestion and endoscopic nasal polyp score on a background of mometasone furoate nasal spray</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

1 No further development has been reported (asthma)
2 Appears to be discontinued for asthma
3 Discontinued (asthma, atopic dermatitis) Refs [1] [2]
4 No further development has been reported (asthma)
5 Appears to be discontinued for atopic dermatitis
6 No further development has been reported

rhIL-4Ra recombinant human IL-4 receptor α, FEV1 forced expiratory volume in one second, SABA short-acting beta agonists, FEVNO fractional exhaled nitric oxide, IV intravenously, SC subcutaneously, ACQ Asthma Control Questionnaire, Q2W every 2 weeks, Q4W every 4 weeks, OCS oral corticosteroids, TCS topical corticosteroids, EASI Eczema Area and Severity Index, IGA Investigator’s Global Assessment, SCORAD SCORing Atopic Dermatitis, NRS numerical rating scale, DLQI Dermatology Quality of Life Index, HADS Hospital Anxiety and Depression Scale, LAR late asthmatic response, EAR early asthmatic response, AHR airway hyperresponsiveness, SDI Straumann Dysphagia Instrument, EoE-ERFS Eosinophilic Esophagitis Endoscopic Reference Score.
AUTHOR DECLARATION FORM

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Journal name: BioDrugs  Corresponding author: Danielle Karo-Atar and Ariel Munitz

Manuscript title: Therapeutic targeting of the IL-4/IL-13 Signaling Pathway: In Allergy and Beyond

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- Approval of the final submitted version of the manuscript.
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☒ The entire content of the manuscript.

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